EVALUATION OF TRIPLE THERAPY VERSUS PROBIOTIC (LACTOBACILLUS REUTERI) - PROTON PUMP INHIBITOR COMBINATION IN ERADICATION OF HELICOBACTER PYLORI

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BAHRIA UNIVERSITY ISLAMABAD
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A thesis submitted in fulfilment of the requirements for the award of the degree of Master of Philosophy (Pharmacology)

DEPARTMENT OF PHARMACOLOGY
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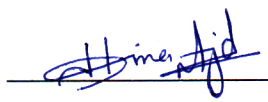
Student / Author's Sign: 

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DEDICATED TO MY PARENTS

For their endless love, support, and encouragement

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ABSTRACT

The efficacy of commonly prescribed conventional triple therapy drugs such as amoxicillin, clarithromycin and proton pump inhibitor for the treatment of peptic ulcer is decreasing gradually due to the development of antibiotic resistance in the body and also due to the adverse effects caused by frequent use of antibiotics. Probiotics have displayed to have positive effects on the eradication rate of *Helicobacter pylori* and prevention of side effects associated with antibiotics. The aim of the study was to evaluate and compare the efficacy and safety of conventional triple therapy versus Lactobacillus *reuteri* + proton pump inhinbitor. The study was conducted in National Medical Center, Karachi on 100 diagnosed patients of peptic ulcer. Male and female patient's ≥ 18 years of age, fulfilling the inclusion criteria were inducted in the study after informed consent. These patients were systematically randomized into 2 groups each having 50 members. Group A received tablet proton pump inhibitor 20 mg twice daily + Clarithromycin 500 mg BD + Amoxicillin 1 gm BD for 14 days and group B received capsule Lactobacillus *reuteri* 100 mg twice daily + proton pump inhibitor 20 mg BD for 14 days. The parameters that were investigated include: Hemoglobin (Hb), Liver function test (LFTs), Renal function test (RFTs) and stool antigen test. For the analysis, descriptive statistics were calculated using SPSS version 23. For comparison of two groups A and B, independent T test was used and for comparison of qualitative variables chi-square test was used. The mean Hb in group A at baseline and after 14 days was same 15.00±1.321 % and mean Hb in group B at baseline and after 14 days was also same 14.94±1.399 %. Further, we have found insignificant mean difference for Hb at baseline and after 14 days between the study groups (p=0.999). The mean ALT in group A at baseline and after 14 days was same 25.67±4.033 IU/L while in group B was also same 25.46±4.037 IU/L and the mean AST in group A at baseline and after 14 days was same 28.83±5.335 units/L while in group B was also same 29.59±4.440 units/L along with insignificant mean difference for ALT and AST at baseline and after 14 days between the study groups (p=0.999). The mean serum urea in group A at baseline and after 14 days was same 15.43±2.208 mg/dL while in group B was also same 15.24±1.923 mg/dL and the mean serum creatinine in group A at baseline and

after 14 days was same 0.83±0.167 while in group B was also same 0.83±0.163 mg/dL along with insignificant mean difference for serum urea and serum creatinine between the study groups (p=0.999). The stool antigen test showed 56.5% positive and 43.5% negative patients in group A while in group B this test showed 34.8% positive and 65.2% negative patients after treatment. When we compared both the groups, we found statistically significant difference between the two groups (p=0.036). Probiotic (Lactobacillus *reuteri*) along with proton pump inhibitor have shown better efficacy and also helped in minimizing the side effects associated with conventional triple therapy.

Key words:

Peptic ulcer, *Helicobacter pylori*, Amoxicillin, Clarithromycin, Proton pump inhibitor, Probiotic (Lactobacillus *reuteri*).

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ALT	Alanine aminotransferase
AST	Aspartate transaminase
BQT	Bismuth Quadruple Therapy
CBC	Complete Blood Count
CTT	Clarithromycin based Triple Therapy
FISH	Fluorescent In Situ Hybridization
GERD	Gastroesophageal Reflux Disease
GLDH	Glutamate dehydrogenase
Hb	Hemoglobin
HPET	H. pylori-eradication Therapy
L. reuteri	Lactobacillus *reuteri*
LFT	Liver Function Test
MALT	Mucosa-Association Lymphoid Tissue
MDH	Malate dehydrogenase
NADH	Nicotinamide Adenine Dinucleotide
NSAIDs	Non-steroidal Anti-inflammatory Drugs
PBPs	Penicillin-Binding-Proteins
PCR	Polymerase Chain Reaction
PPI	Proton Pump Inhibitors
PU	Peptic Ulcer
RCT	Randomized Controlled Trial
RFT	Renal Function Test
SMT	Standard Management Therapy
STT	Standard Triple Therapy
WHO	World Health Organisation

CHAPTER 1

INTRODUCTION

1.1 Peptic Ulcer (PU)

Peptic ulcer is the disorder of gastrointestinal tract. It is mainly categorized by the termination of the internal lining in the digestive tract due to the increased secretion of the pepsin. It commonly happens in the stomach and proximal region of duodenum. The stomach is the organ which plays a very important part in the ingestion of foods that we eat daily. The stomach also resists multiple damaging elements like hydrochloric acid, alcohol and refluxed bile salts which can damage the barrier of gastric mucosa and cause injury to the epithelial cells in the stomach. Gastrointestinal disorders like indigestion, GERD, peptic ulcers, irritable bowel syndrome, ulcerative colitis and inflammatory bowel disease have become very common now a day, with at least 60% of the population is affected once every year (Pathak *et al.,* 2016*).*

Gastric ulcers are mostly present in the lesser curvature and duodenal ulcers are present most commonly in the first part of the duodenum. Gastric ulcers have the potential to cause gastric cancer but the duodenal ulcers do not have cancerous risk. Gastric carcinoma is categorized as the third most common cancer globally and those persons who are mostly infected from this cancer belongs to countries like China, Japan and Korea. It has very poor prognosis with only 1 in 5 persons are able to survive more than 5 years after getting diagnosed with gastric cancer (Hooi *et al.*, 2017). A newer technique used for early diagnosis and staging of gastric cancer is called “Endoscopic Ultrasonography” (Kulshreshtha *et al.,* 2017). Duodenal ulcers are caused due to alterations in the regulation of acid secretions by *H. pylori* and also alterations in the duodenal bicarbonate secretions due to mucosal damage (Graham, 2014). The duodenal ulcers are more prevalent than the gastric

ulcers and it is more common in males than females. Duodenal ulcers are found to be more dominant than the gastric ulcers and the projected occurrence of these ulcers were 32:1 and 5:1 in India and Pakistan respectively (Lakhani *et al.*, 2015). Patient presented with duodenal ulcers have symptoms like they will feel starving or have stomach pain at nighttime. While on the other hand, patients presented with gastric ulcers will have symptoms like postprandial abdominal pain, nausea, vomiting and weight loss (Tsai & Brooks, 2019). Both these ulcers are linked with stomach bleeding, damages and blockage which leads to the increase in death rates (Sadeeqa & Anwar, 2018). The difference between gastric and duodenal ulcers are given in table 1.1.

The peptic ulcer is divided into following types on the basis of the location of ulcers (Tsai & Brooks, 2019):

- Type 1: It is mostly present in the gastric region and near the lesser curvature of the stomach. They are very common and consist of almost 60% of the gastric ulcer. These type of ulcers are mainly caused by *H. pylori* infection.
- Type 2: These types of ulcers are present in the body of the stomach combined with duodenal ulcers.
- Type 3: These ulcers are present in the prepyloric region. They are characterized by increase secretion of acid.
- Type 4: These ulcers are present in the proximal region of the stomach or cardia. They are characterized by low secretion of acid.
- Type 5: These types of ulcers are present throughout the stomach and are mostly due to prolong use of NSAIDs or steroids (Tsai & Brooks, 2019).

The patient suffering from peptic ulcer disease presents with following clinical symptoms which are as under (Roy, 2016):

1. Loss of appetite
2. Mild nausea
3. Bloating
4. Epigastric pain

5. Abdominal rigidity
6. Bleeding
7. Dark or black stool (due to bleeding)
8. Tachycardia
9. Perforation
10. Stomach outlet obstruction

1.1.1 Prevalence of Peptic Ulcer

It is a universal problem with a lifetime risk of expansion ranging from 5% to 10% (Narayanan *et al*., 2018). It is one of the increasing problems which is seen in overall the world in every age groups (Sadeeqa & Anwar, 2018). Every year about 4 million people are affected by peptic ulcer disorder in the whole world. The countries with the highest prevalence of peptic ulcer disease are Africa (79.1%), Latin America and Caribbean (63.4%) and Asia (54.7%) (Hooi *et al*., 2017). The history of peptic ulcer dates back to the 16th century. In 1679, Bauhin suggested that infection of the stomach led to a gastric ulcer which then ruptured. The first person who had peptic ulcer disease was from the Western Han dynasty and he died in 167 BC. His autopsy was done which showed perforated prepyloric ulcer causing diffuse peritonitis and disseminated coagulopathy (Graham, 2014). The first identified case of abdominal hemorrhage was presented in 1704. Peptic ulcer disorder was the basis of obstruction in 62% of patients from the year 1962 to 1975 and in 45% of patients from the year 1975 to 1985. (Gibson *et al*.) inspected that about 33% of individuals with peptic ulcer disorder and gastric obstruction were *H. pylori* positive (Kulshreshtha *et al*., 2017). In 1983, Marshall and Warren discovered the *H. pylori* bacteria for the first time by conducting a probable study of 100 patients who underwent endoscopy to compare the digestive mucosal biopsy results with clinical and endoscopy data (Lanas & Chan, 2017).

Table 1.1: Distinguishing features of the two major forms of peptic ulcer (Graham, 2014).

S.No.	Features	Duodenal ulcer	Gastric ulcer
1	Incidence	Four times common than gastric ulcers Usual age 25–50 years More common in males than in females (4:1)	Less common than duodenal ulcers Usually beyond 6th decade More common in males than in females (3.5:1)
2	Etiology	Most commonly as a result of *Helicobacter pylori* infection. Other factors are hypersecretion of acid-pepsin, alcoholic cirrhosis, tobacco, chronic pancreatitis, blood group O, genetic factors, etc.	Gastric colonization with *H. pylori* asymptomatic but higher chances of development of duodenal ulcers. Disruption of mucus barrier most important factor. Association with gastritis, bile reflux, drugs, alcohol, and tobacco.
3	Complications	Commonly, hemorrhage, perforation, sometimes obstruction, is observed. However, malignant transformation never occurs.	Perforation, hemorrhage and at times obstruction, are common. Malignant transformation less than 1% cases.
4	Clinical features	Pain food relief pattern Night pain common No vomiting No loss of weight No particular choice of diet	Food pain pattern No night pain Vomiting common Significant loss of weight Patients choose bland diet devoid of fried food, curries etc.

Peptic ulcer is a chronic disease with a lifetime prevalence expected to be about 5-10% and frequency 0.1-0.3% annually (Lanas & Chan, 2017). The prevalence rate differs in the world population and the mean age of people which can develop this disease is between 30 to 60 years. But it is also found that this disease can happen at any age. The peak incidence of age of developing gastric ulcer is found to be between 55-65 years and the peak age of people who are infected with duodenal ulcer is around 45 years (Azhari *et al*., 2018). Approximately 500,000 persons in the United States develop this disease every year and mostly it occurs between the age of 25 and 64 years (Kulshreshtha *et al*., 2017). In Canada the frequency of peptic ulcer is expected to be around 30% (Thung *et al*., 2016). In European countries the rate of death from gastric ulcers headed that from the duodenal ulcers. It is supposed that *H. pylori* disease is more prevalent in developing countries than developed ones (Lakhani *et al*., 2015).

In Asia, a steady decline has been seen in the occurrence of peptic ulcer disorder in different ethnic groups including Malay, Chinese and Indian population in the previous 20 years (Lanas & Chan, 2017). Between East Asian countries, the incidence rate of *H. pylori* infection noted was 58.07% in China, 39.3% in Japan, 59.6% in South Korea, 35.9% in Malaysia, 31% in Singapore and 57% in Thailand. The highest frequency of gastric carcinoma due to *H. pylori* infection was reported from Asia (John B *et al*., 2017). The frequency of *Helicobacter pylori* is greater in emerging countries among the people with a low socio-economic status. Pakistan is one of the developing countries in South Asia with common *H. pylori* infections. In Pakistan, the occurrence of peptic ulcer disorder mainly caused by *Helicobacter pylori* bacteria is found to be 85.1% (Hussain *et al*., 2012).

1.1.2 Pathophysiology of Peptic Ulcer

The pathophysiology of peptic ulcer is attributed mainly due to the imbalance between aggressive agents like gastric acid, pepsin and *H. pylori* infection and mucosal protective agents like bicarbonate, mucus and prostaglandins (Ankita *et al*., 2017). The factors involved in the pathogenesis of peptic ulcer are shown in figure 1.2.

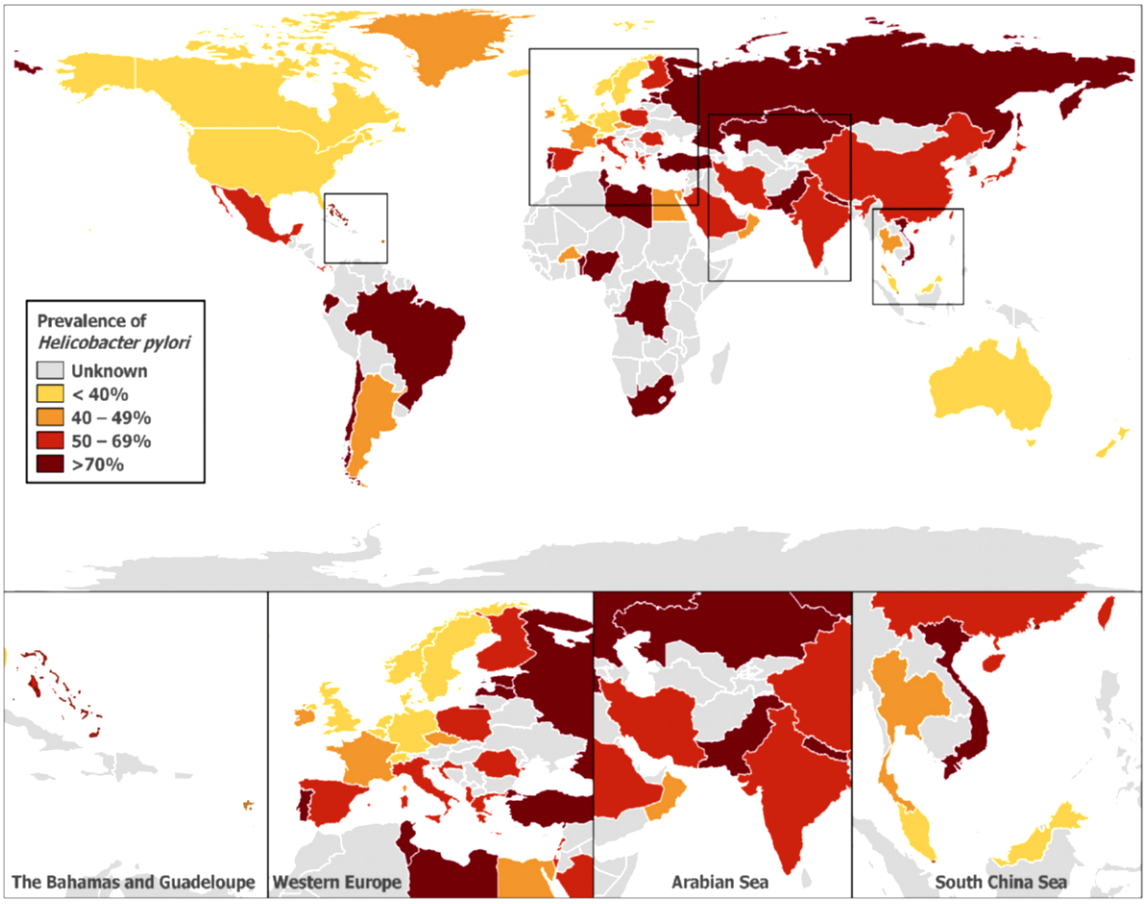


Figure 1.1: Global prevalence of HP choropleth map. Certain regions are magnified to better display the smaller countries (Hooi *et al.*, 2017).

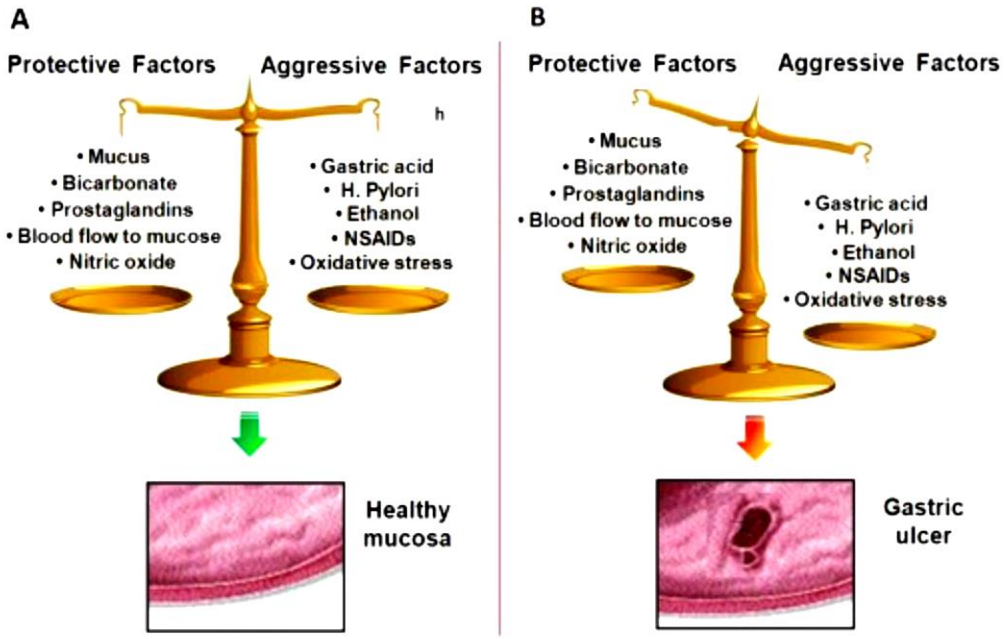


Figure 1.2: Factors involved in the pathogenesis of peptic ulcer (A) and (B) (Ankita *et al*., 2017).

1.1.2.1 Helicobacter Pylori Infection

Helicobacter pylori is a gram negative micro-organism which resides in the host intestinal mucosa. In 1994, the United States National Institutes of Health Consensus Development Panel concluded that *H. pylori* bacteria contributes a major role in the pathogenesis of peptic ulcer disorder (Rashid *et al*., 2016). This bacterium has been recognized as the chief contributing reason in the expansion of duodenal and gastric ulcers. Studies from the 1980s and 1990s showed that it is the sole reason for producing 70% of gastric ulcers and 90% of duodenal ulcers (Kulshreshtha *et al*., 2017).

The *H. pylori* bacteria have a unique feature like urease production which allows it to stay alive in the harsh environment of the stomach. The process by which these bacteria causes disease can be divided into different steps. In the initial stage, the microbe disturbs the antibacterial action of stomach acid barrier, it enters the mucus layer and gets used to of the environmental settings of digestive mucus. In the second stage, bacteria stick to the stomach mucosa of the host and activates the manifestation of different microbial genes which permits the harmful bacteria to survive in this situation and avoid removal which occurs due to peristalsis of the mucous layer. *Helicobacter pylori* settlement has a unique factor that is enzyme urease, which causes conversion of urea to produce by-products like ammonia and carbon dioxide which increase the pH and bring it to neutral which protects the cell of bacteria from stomach acid (Espinoza et al. 2018). *H. pylori* infection is categorized by penetration of the inflammatory cells within the intestinal mucosa. *Helicobacter pylori* produces protease and lipase which is accountable for destruction of stomach mucus and cell damage from backward flow of gastric acid. Patients with *H. pylori* disease produces lower quantity of gastric acid maybe due to apoptosis prompted by pro-inflammatory mediators. *H. pylori* infection may also increase acid secretion in the stomach. Raised gastric secretion enhances the duodenal acid load, which results in mucosal damage and formation of ulceration. *H. pylori* also comprises some of the most harmful virulence genes that is vacuolating cytotoxin (VacA) and cytotoxin-

associated gene A (CagA) which are linked with peptic ulcer disease (Ahmad *et al*., 2019). The role of *H. pylori* and the secretion produced during the pathogenesis by *H. pylori* is shown in figure 1.3.

1.1.2.2 Non-steroidal anti-inflammatory drugs (NSAIDs)

After Helicobacter pylori infection, NSAID and aspirin is the second most common cause which contributes in the pathogenesis of peptic ulcer disorder (Narayanan *et al*., 2018). It is also the most common cause of gastric mucosal injury in Pakistan (Rashid *et al*., 2016). The mechanism by which NSAID produce peptic ulcer is it blocks the synthesis of prostaglandin by obstructing COX-1 enzyme which results in a reduction in gastric mucus and bicarbonate production (Soreide *et al*., 2015). Prostaglandins plays an important role in defense mechanism and repair process. They enhance the production of mucus which acts as a protective barrier of intestinal epithelium and it also increase bicarbonate production that lowers the gastric acid secretion, thus maintain the sufficient blood flow in mucosal microcirculation. Prostaglandins decrease the stimulation of mast cells plus prevent leukocyte attachment to vascular endothelium. NSAIDs also stimulates gastric acid secretion by inhibiting somatostatin release, which inhibits the acid secretion (Ahmad *et al*., 2019). The role of NSAIDs in the pathogenesis of peptic ulcer disease is shown in figure 1.4.

1.1.2.3 Gastric acid and Pepsin

Stomach acid and pepsin secretion plays an important part in damaging the mucosa. Digestive acid is a fluid which is produced in the stomach and it triggers the digestive enzymes which helps in the ingestion of proteins. The acid is produced by gastric parietal cells and has a pH of 1.5-3.5 in the lumen of the stomach. Patients

who are diagnosed with *H. pylori* produces less amount of gastric acid than normal because of pro-inflammatory mediator induced apoptosis. However, *H. pylori* infection also causes an increased secretion of gastric acid. This increase secretion of gastric acid enhances the duodenal acid which causes mucosal damage and ultimately leads to ulceration (Zatorski, 2017). Pepsinogen which is the precursor of pepsin is released by the chief cells present in the body and fundus of the stomach. Pepsin is an important digestive protease found in the gastric juice. It is triggered by acid having pH 1.8 to 3.5 and deactivated at pH 4 reversibly, and irreversibly at pH 7. It has a proteolytic activity and also found to help in ulcer formation (Ankita *et al.*, 2017).

1.1.2.4 Mucosal Defense and Repair

The mucosal defense and healing mechanism protects the stomach from toxic endogenous and exogenous substances. The primary line of mucosal protection comprises of "mucus-bicarbonate-phospholipid barrier". The mucosa of stomach is surrounded by a cover which is made up of mucus gel and bicarbonate anions. This cover maintains the pH near to 7 and also has the capability to retain the bicarbonate anions. The bicarbonate secretion is critical to sustain a pH gradient at the epithelial surface which is a main line of protection against stomach acid. Whenever, this protective barrier is damaged other compensatory mechanism are activated. These mechanisms include intracellular acid neutralization, rapid epithelium renewal and maintenance of mucosal blood flow. The gastric mucosa also produces prostaglandins, mainly PGE_2 and PGI_2, which protects and maintains the mucosal integrity. Prostaglandins also have potential to produce bicarbonate, stimulate mucus, reduce acid secretion and enhance mucosal blood flow (Perico *et al.*, 2020).

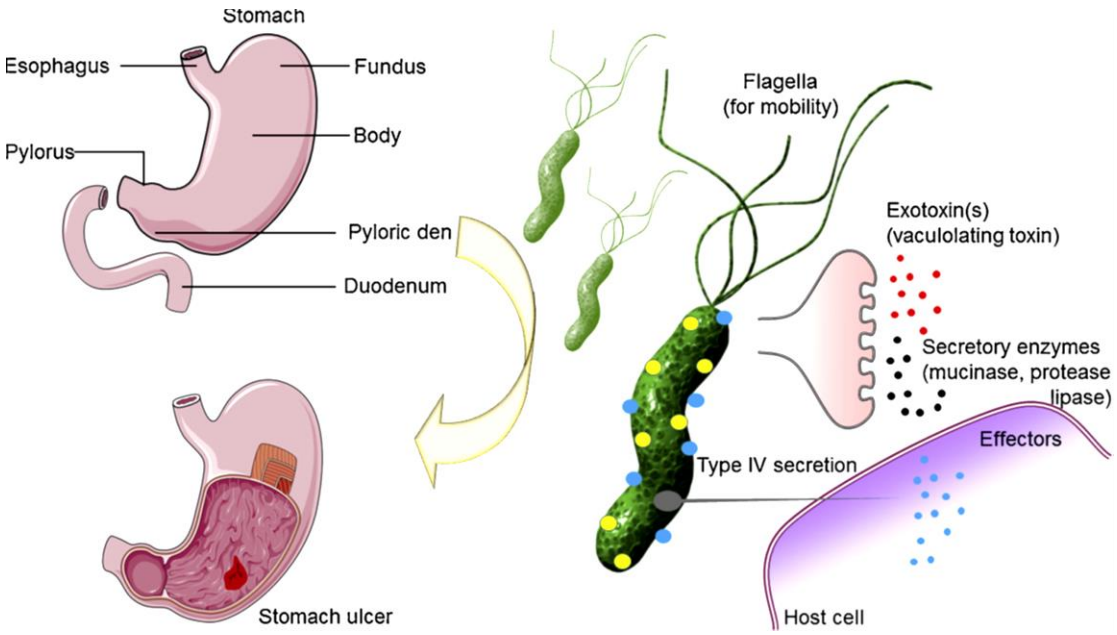


Figure 1.3: The role of *H. pylori* and the secretions produced during the pathogenesis by *H. pylori* (Ahmad *et al*., 2019).

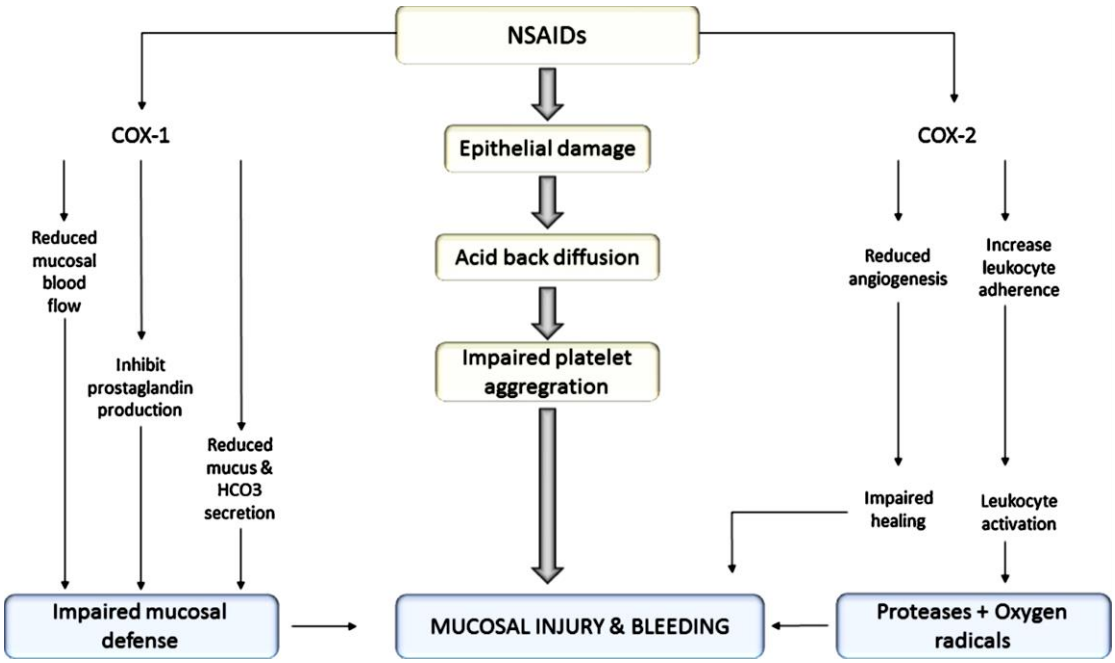


Figure 1.4: Mechanism involved in pathogenesis of peptic ulcer by nonsteroidal anti-inflammatory drugs (Narayanan *et al*., 2018).

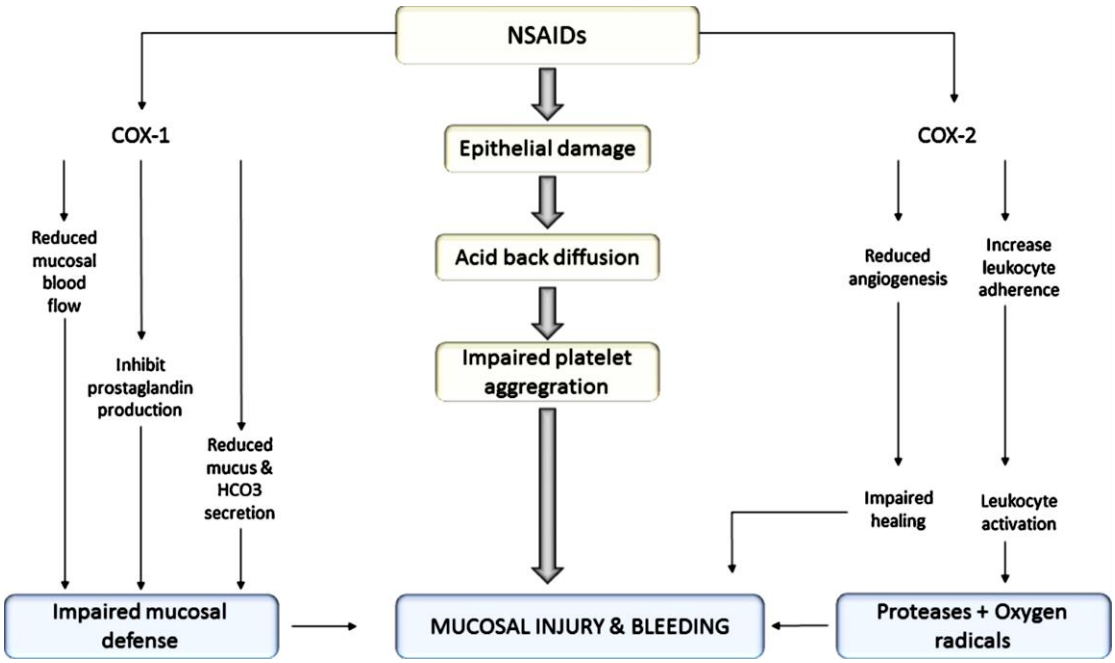


Figure 1.4: Mechanism involved in pathogenesis of peptic ulcer by nonsteroidal anti-inflammatory drugs (Narayanan *et al*., 2018).

The second line of defense is made up of continuous layer of epithelial cells which are connected by tight junctions. These junctions form an impermeable barrier which stops the backward flow of stomach acid and pepsin and protects the stomach lining from damage. These epithelial cells also produce cathelicidins and beta defensins, both contains antimicrobial properties and prevent the gastric mucosa from microbial colonization. The integrity of stomach epithelial cells is sustained by continuous process of cell renewal by mucosal progenitor cells. Proper mucosal flow of blood is very important in delivering the substances like nutrients and oxygen and to eliminate harmful products from stomach mucosa. The proper blood flow is maintained by nitric oxide and prostacyclin, both are potent vasodilators, which are produced from endothelial cells in the stomach. If the proper blood flow is not maintained it will result in tissue necrosis. (Zatorski, 2017).

1.1.3 Etiology of Peptic Ulcer

The key part in the growth of peptic lesion is performed by *Helicobacter pylori* bacteria, NSAIDs, gastric acid and pepsin. However, excessive alcohol drinking, smoking tobacco, eating unhealthy food and emotional stress are also essential etiological factors related to the peptic ulcer disease (Perico *et al*., 2020).

1.1.3.1 Helicobacter Pylori

Almost half of the global populace is infested by *Helicobacter pylori* bacteria, due to this reason it is the main causative factor of peptic ulcer disorder (Kuna *et al*., 2019). It is a gram negative, helix shaped bacteria which is present in the mucus layer at the top of the gastric epithelium. The association between peptic ulcer and *Helicobacter pylori* infection is discovered by Marshall and Warren in 1983 (Narayanan *et al*., 2018) and for this discovery these two scientist were awarded the

Nobel Prize in 2005 (Roy, 2016). If *H. pylori* is not treated early it can lead to gastric adenocarcinoma and gastric-MALT lymphomas (karakus *et al*., 2018).

1.1.3.2 Non-steroidal Anti-inflammatory Drug (NSAIDs)

NSAIDs is the famous etiological factor of peptic ulcer disease. Also, cases of NSAIDs induced peptic ulcer are increasing because of the increase in the cardiovascular disease in the aging population globally (Lee *et al*., 2017). These drugs block the function of cyclo-oxygenase enzyme which inhibits the conversion of arachidonic acid to prostaglandins which will lead to the damage of gastric mucosa (Pathak *et al.* 2016).

1.1.3.3 Smoking

Smoking increases the risk of peptic ulcer disease. It also impairs the healing of duodenal and gastric ulcers. Smoking increases the formation of stomach acid and decrease the bicarbonate production (Soreide *et al.*, 2015). It also inhibits the epithelial cell renewal in the abdomen and alters the immune system. Therefore, it increases the incidence and recurrence of peptic ulcer disease (Ankita *et al*., 2017).

1.1.3.4 Alcohol Consumption

Alcohol consumption causes irritation of the gastric mucosa and it also causes damage to the mucosal barrier which enhances the permeability of gastric mucosa (Perico *et al*., 2020). It increases the gastric acid secretion and decrease the prostaglandin formation which will result in mucosal injury. High doses of alcohol slows down the bowel movements and delays the gastric emptying (Zatorski, 2017).

1.1.3.5 Dietary Factors

Nutritional deficiency has been regarded as one of the etiological factor in the progression of peptic ulcer disorder. Gastric ulcer is found more in people who belongs to low socioeconomic status. Drinking coffee is found to be linked with peptic lesion and gastroesophageal reflux disease (GERD). Coffee contains peptides that stimulate the release of gastrin, which triggers the production of gastric acid (Chung KT *et al*., 2017). Spices like red chilies act as an aggravating factor in peptic ulcer. It increases the symptoms of disease. These spices can cause indigestion and reflux of stomach acid especially in those patients who already have the disease that is why it is advised to avoid red chilies in meal. But the actual role of red chillies in causing peptic ulcer disease is still controversial and under evaluation (Kulshreshtha *et al*., 2017).

1.1.3.6 Genetic Factors

Genetic predisposition is also considered as one of the etiological factor of peptic ulcer disorder. Close relatives who have gastric ulcers are at three times more danger of developing gastric ulcers than duodenal ulcers. Increased frequency of blood group O and A along with nonsecretor status have been related as a threat for peptic ulcer disease (Kulshreshtha *et al*., 2017).

1.1.4 Complications of Peptic Ulcer

Patient with peptic ulcer will have complications like stomach bleeding, perforation, penetration to a surrounding organ and obstruction from fibrotic

stricturing. Therefore, complete abolition of *Helicobacter pylori* might help in the treatment of these complications (Sverdén *et al*., 2019).

1.1.4.1 Bleeding

It is a very common complication of peptic ulcer disorder and it includes around 10-20% of individuals (Me *et al.,* 2015). It is also the most frequent cause of emergency and requires hospitalization. Stomach bleeding occurs in 19 to 57 per 100,000 individuals annually, according to systemic evaluation (Kirsch & Hirsch-Reilly, 2017). It is also the most common cause of upper gastrointestinal bleeding which occurs due to frequent use of NSAIDs (Laine, 2016). The treatment of bleeding ulcer can be done by maintaining the vital signs, eliminating the causative factors like NSAIDs and by prescribing the proton pump inhibitors (Bang & Baik, 2016).

1.1.4.2 Perforations

It occurs in about 5% of patient who are suffering from peptic ulcer disease. It mostly occurs in anterior wall of the duodenal bulb in 60% cases of duodenal ulcers and in the lesser curvature side of gastric body mostly in 40% cases of gastric ulcer. The patients will complain of sudden and severe abdominal pain in the epigastrium which may radiate to other parts of the body (Me *et al.*, 2015). The diagnosis of perforation should be done by taking proper history, doing physical examination and by detecting free air in X-ray. The treatment of perforation includes saline infusion, nasogastric tube drainage, by prescribing drugs like proton pump inhibitors and broad-spectrum antibiotics and by doing surgery. Perforation is comparatively not very common, happening in 4 to 14 per 100,000 persons annually.

Around 8.6% of individuals with peptic ulcer bleeding and 23.5% of persons with perforation cannot survive more than 30 days (Chung KT *et al.*, 2017).

1.1.4.3 Penetration

It mostly occurs when ulcer progress towards the wall of the stomach or duodenum. It mostly involves 25% of duodenal ulcers and 15% of gastric ulcers (Me *et al*., 2015). Patient may present with symptoms like severe radiating pain to the back which sometimes awake patient at night and also does not go away after eating. The ulcer can penetrate into adjacent organs like pancreas, liver, colon and biliary tract. To diagnose how much ulcer has been penetrated the endoscopic or radiologic examination can be very helpful. This can be treated by eradicating *H. pylori* bacteria, stopping causative factors like NSAIDs, by using anti-peptic ulcer medications and if the penetration is worse and cannot be treated by these methods then the surgical management is the only option (Bang & Baik, 2016).

1.1.4.4 Gastric Outlet Obstruction

It is a very serious and uncommon complication of peptic ulcer disease. The obstruction commonly take place in the prepyloric region, duodenal bulb or post-bulbar region of the intestine. The obstruction usually develops by inflammation, edema and fibrosis from the chronic ulcers. The patients usually present with symptoms like nausea, vomiting, early satiety and anorexia. For the diagnosis of obstruction endoscopy and computed tomography can be used. For the treatment of obstruction, balloon dilatation method by using endoscopic instrument is used. To prevent the reoccurrence of stenosis, steroid injections can be given by using endoscopic ultrasound. Other treatment options are eradication of *H. pylori*, by using

medications like proton pump inhibitors and by stopping contributing factor like NSAIDs (Bang & Baik, 2016).

1.1.5 Diagnosis of Peptic Ulcer

The identification of peptic ulcer disorder is done on the basis of clinical presentation and by doing definite tests. The diagnostic test for the identification of *H. pylori* disease can be categorized into non-invasive and invasive type. The diagnostic test for *Helicobacter pylori* infections includes: fecal antigen test, serology test, urea breath test, rapid urease test or gastric biopsies obtained at the time of upper endoscopy and serological tests (Kavitt *et al.*, 2019).

1.1.5.1 Urea Breath Test

In this test urease activity is checked which is produced by *H. pylori* bacteria. This test is performed by using either non-radioactive C^{13} or radioactive C^{14} isotope (Thung *et al*., 2016). This test has 100% specificity and sensitivity approach. The advantage of using this test is it has ability to monitor eradication of *H. pylori* after completion of treatment. This test is inconvenient and costly (Fashner & Gitu, 2015).

1.1.5.2 Stool Antigen Test

In this test feces examination is done to detect the existence of *H. pylori* antigen by using monoclonal and polyclonal anti-*H. pylori* antibodies (Pathak et al., 2016). It only detects active infections and it can be used as an alternative to urea breath test. This test is cheaper and more convenient than urea breath test (Fashner & Gitu, 2015).

1.1.5.3 Serologic Test

In this test immunoglobulin G antibody is detected in serum to confirm the diagnosis of *H. pylori*. This test is useful in detecting active infections and also in mass population surveys (Fashner & Gitu, 2015).

1.1.5.4 Upper Endoscopy

Esophago-gastro-duodenoscopy (EGD) is the gold standard test which is used for the first line of investigation and the specificity and sensitivity of this test in diagnosing gastric and duodenal ulcers is up to 90% (Roy, 2016). This test is also used to rule out cancer or other malignancies in individuals who have 55 years of age or older (Thung *et al*., 2016).

1.1.5.5 Histology

This test is used to detect the *H. pylori* bacteria and other malignancies like intestinal metaplasia, atrophy, chronic inflammation and aggregation of lymphoid.

The staining of biopsy specimen is done by using different staining solutions (karakus *et al*., 2018).

1.1.5.6 Culture

This test is also performed for the identification of *H. pylori* disease. There is different culture medium which are used for the isolation, identification and antibiotic testing of bacteria from the samples which are obtained through biopsy. The CIM medium is the best solution for the culture of *H. pylori* in solid media (karakus *et al*., 2018). The summary of the advantage and disadvantage of all the diagnostic test is shown in table 1.2.

1.1.6 Treatment of Peptic Ulcer

The treatment of peptic lesion has now become curable by effective annihilation of the *H. pylori* bacteria. However, management of peptic ulcer bleeding remains a critical clinical challenge.

At the time of 90s era, the conventional triple therapy was considered the benchmark in the cure of peptic ulcer disorder. The conventional triple regimen comprises of a proton pump inhibitor, clarithromycin and amoxicillin or metronidazole. This triple therapy was recommended at the first Maastricht conference in 1996 and has been widely used for twenty years. As the cases of peptic ulcer are increasing at an alarming rate this can cause more financial burden on the healthcare system of our country and also due to costly medicines and relapse of H. pylori bacteria, this disease causes a huge dent on the economic situation of the person and make the treatment more burdensome (Zhang *et al*., 2017).

Table 1.2 Diagnostic Tests for *H. pylori* (Kavitt *et al*., 2019)

TEST	SENSITIVITY	SPECIFICITY	ADVANTAGES	DISADVANTAGES
Urea Breath Test	95%	96%	Confirms cure	Accuracy affected by PPI and antibiotic use
Fecal Antigen Test	95%	94%	Confirms cure	Accuracy affected by PPI and antibiotic use
Serology	85-92%	79-83%	Only test not influenced by PPI or antibiotic use	Cannot confirm cure
Histology	>95%	>95%	Permits visualization, confirms cure	Requires endoscopy, affected by PPI and antibiotic use.
Culture	70-90%	100%	Allows determination of antimicrobial sensitivity, confirms cure.	Requires endoscopy, result takes several days, affected by PPI and antibiotic use.

The increase in the incidence of resistance to these drugs especially to the main antibiotics, clarithromycin and metronidazole has reduced the effectiveness of standard therapy. In a latest systematic analysis, the worldwide prevalence of Helicobacter pylori resistance to drug clarithromycin has been suggested to be more than 17.2% displaying a growth universally (Goderska et al., 2018).

The resistance to clarithromycin occurs due to point mutation in the area of peptidyl-transferase in the domain V of 23S rRNA it will result in the disability of adhesion between the drug and the subunit of ribosome (Eslami *et al*., 2019).

Metronidazole is also an important component in the triple therapies which is also related to a high level of resistance. The resistance to metronidazole is very high in developing countries which is reported to be between 50 to 100%. The incidence of metronidazole resistance in Europe is estimated to be about 17% and in America it is about 44%. The highest prevalence of resistance is found in Africa which is estimated to be about 90% (Goderska *et al*., 2018). This drug also induces several side effects like nausea, vomiting, headache and metallic taste. These adverse effects can cause poor patient compliance (Jung *et al*., 2018). The mechanism of resistance of metronidazole is mutation in the enzyme NADPH nitroreductase (RdxA), NADPH-flavin-oxidoreductase (FrxA) and ferredoxinlike enzymes (FrxB). The virulence strains which is devoid of cagA gene are more resistant (Eslami *et al*., 2019).

Amoxicillin is a beta lactamase antibiotic, it works by hindering the formation of peptidoglycans mainly by regulatory the enzyme such as penicillin-binding-proteins (PBPs) which will prevent the formation of microbial cell wall and results in bacterial death. Mutation in the PBP1 gene will cause resistance to amoxicillin. But luckily the resistance to amoxicillin is very low (Eslami *et al*., 2019).

Proton pump inhibitors are the popular and effective medicines for the management and prophylaxis of peptic ulcer. These are the most frequently used and prescribed medicines in the world nowadays (Kavitt *et al*., 2019). Proton pump inhibitors include omeprazole, lansoprazole, pantoprazole, rabeprazole and

dexlansoprazole. Its mode of action is; it reduces the formation of stomach acid by attaching irreversibly to the hydrogen/potassium ATPase enzyme found on the parietal cells of stomach. Proton pump inhibitors have very short half-life approx. 1 to 2 hours. They are highly protein bound drugs and undergoes metabolism by hepatic P450 cytochrome enzyme. These drugs are mainly excreted through renal route (strand *et al.*, 2017). Apart from eradicating the *H. pylori* bacteria, these drugs are useful in the management of nonerosive reflux disorder, esophagitis, Zollinger-Ellison syndrome, deterrence of NSAID induced gastroduodenal ulcers and functional dyspepsia. The adverse effects of chronic use of this drug includes: headache, nausea, diarrhea, constipation, stomach discomfort, intestinal inflammation and vitamin B12 malabsorption (Horvath *et al.*, 2020).

Because of increased level of resistance and side effects of the two main antibiotics (clarithromycin and metronidazole) substitute approaches are being employed in medical practice to cure the *Helicobacter pylori* resilient strains. When the first-line remedy fails to cure the disease then alternate drugs are suggested and it should not include metronidazole or clarithromycin (Malfertheiner & Schulz, 2020).

1.1.6.1 Bismuth quadruple therapy

The latest Maastricht V/Florence consensus 2016 suggested the bismuth-containing quadruple treatment as a main line therapy in those regions where resistance to clarithromycin is very high. This therapy consists of dual antibiotics, tetracycline and metronidazole plus bismuth and proton pump inhibitor for a period of 14 days (Song *et al.*, 2018). But the bismuth salts are very toxic and most of the patients cannot tolerate this drug. That is why this therapy is not very effective in some countries. Tetracycline is a protein synthesis inhibitor it works by interfering with 30S subunit of the ribosome and also inhibits the function of amino acid transferase. The resistance to tetracycline develops because of mutation in the 16S rRNA gene. But luckily the resistance to tetracycline is very low less than 2% (Eslami *et al.*, 2019).

1.1.6.2 Non-bismuth quadruple therapy

This therapy includes drugs: proton pump inhibitors, clarithromycin, amoxicillin and metronidazole for ten days. This regimen is also given in those parts where resistance to clarithromycin is very high. But the main disadvantage of this therapy is it contains a lot of drug combination as compared to other therapies which will result in poor patient compliance (Goderska *et al*., 2018).

1.1.6.3 Levofloxacin-based therapy

Levofloxacin is a broad spectrum quinolone and it works by interfering with DNA *gyrA* and *gyrB*. When there is point mutation in *gyrA*, then resistance to quinolone develops. This therapy has replaced clarithromycin in standard triple regimen (Eslami *et al*., 2019). This therapy is given in those areas where resistance to clarithromycin and metronidazole is at peak. But high development of resistance to fluoroquinolone is also found in some areas. Due to this reason, this remedy is reserved as second-line treatment choice. This regimen contains drugs: levofloxacin, proton pump inhibitor and amoxicillin for a duration of 14 days. The efficacy of this therapy is between 74 to 81% (Kuna *et al*., 2019).

1.1.6.4 Salvage therapy

This regimen is considered when all the recommended treatment options have failed to cure the infection. This therapy is also called “rifabutin-based triple therapy” and this regimen contains drugs like: proton pump inhibitor (PPI), rifabutin and amoxicillin for 10 days (Kuna *et al*., 2019). It is an anti-tuberculosis drug which works by inhibiting the beta subunit of RNA dependent DNA polymerase of *H. pylori* bacteria which is coded by gene called *rpoB*. Resistance develops due to mutation in *rpoB* gene. The efficiency of this regimen is 66 to 70% (Eslami *et al*., 2019). The efficiency of all the *H. pylori* eradication treatment regimens is shown in table 1.3.

Table 1.3: Types and efficiency of *Helicobacter pylori* eradication treatment options (Kuna *et al*., 2019).

TYPE	DURATION	EFFICIENCY
First Line: **Standard Triple Therapy:** PPI + Two antibiotics (Clarithromycin + metronidazole or amoxicillin)	7-14 days	70-85%
Second Line: **Bismuth-containing quadruple therapy** PPI + bismuth salts + tetracycline + metronidazole	14 days	77-93%
Non-bismuth based concomitant therapy: PPI + clarithromycin + amoxicillin + metronidazole	14 days	75-90%
Levofloxacin triple therapy: PPI + amoxicillin + levofloxacin	14 days	74-81%
Salvage regimens: **Rifabutin-based triple therapy:** PPI + rifabutin + amoxicillin	10 days	66-70%

1.1.6.5 Probiotics

Probiotics have displayed to have positive effects on the abolition rates and prevention of side effects associated with antibiotics (Pohl *et al*., 2019). Research in the field of probiotics as a new treatment drug is still going on but at a slow pace and only started after 2000. They have confirmed to be helpful in decreasing the side effects of antibiotics and improve the patient compliance. They are widely used in the gastroenterology including using as an adjuvant for the cure of *Helicobacter pylori* infections (Dore *et al.*, 2019).

In 1998, an experiment was conducted by Elliott *et al.* from where the idea of using probiotics came. In a mouse model stomach ulcer was induced by using acetic acid, as a result accumulation of *H. pylori* bacteria occurred quickly at the site of ulcers and impaired ulcer healing but after the administration of probiotic strains Lactobacillus enhanced pace of healing of ulcers occurred. According to history, the idea of using probiotics started about 1900 by the Nobel award winning scientist Elie Metchnikoff. He found that when the live bacteria like (Lactobacillus *bulgaricus*) was consumed it recovers the overall health of the digestive tract (Khoder *et al*., 2016).

The Food and Agriculture Organization and the International Scientific Association for Probiotics and Prebiotics defines probiotics as live microorganisms which when administered in adequate amount confer a health benefit on the host. There are many species of microbes which are present in the stomach for example there are 52 species of Lactobacillus bacteria and 30 species of Bifidobacterium. The mainly studied probiotics used for treating stomach disorders are lactic acid bacteria. These bacteria are not very harmful and they can survive the tough surroundings of the digestive system (Ameen *et al*., 2019).

Probiotics functions through numerous mechanisms to repair the stomach mucosa which is destroyed by peptic ulcer disorder. They work by decreasing the gastric pH and they also secrete antimicrobial agent which fights with pathogenic bacteria to prevent adhesion of *H. pylori* bacteria. They also stop the growth of *H.*

pylori bacteria by releasing the short chain of fatty acids which are produced by the metabolism of carbohydrate and as a consequence the gastric pH will be lowered. Probiotics also increase the production of mucin which will protect the gastric mucosa from damaging and they also help in the maintenance of gastric mucosal membrane. Probiotics also interferes with the host immune system by interrelating with the epithelial cells and decreasing the release of proinflammatory cytokines which will reduce the inflammation of the stomach. Lactobacillus *salavaris* decrease the secretion of interleukin IL-8 and Lactobacillus *acidophilus* inhibits the Smad7 and NF-kB nuclear factor induced by *H. pylori* (Lau *et al*., 2016). Probiotics may also weaken the hypochlorhydria linked with *Helicobacter pylori* infection by discharging lactic acid. They are also capable of fighting with the harmful bacteria for host surface receptors and in this manner prevent the accumulation of pathogens to epithelial cells. Probiotics may function as either bacteriostatic or bactericidal drug (Boltin, 2016). Lactobacillus *reuteri* has an ability to produce reuterin, which is a wide spectrum antibiotic to stop the action of *H. pylori* infection and it also prevents the *H. pylori* bacteria to settle in the digestive mucosa of human and cause damage to the intestinal mucosa (Muresan *et al*., 2019). The summary of overall mechanism of probiotic bacteria is shown in figure 1.5.

Probiotics have displayed to return the microflora to a more beneficial and biological equilibrium in an individual. A lot of studies have reported numerous favorable outcomes of lactobacilli such as destruction of harmful microbes in the intestine and reduction of hypersensitive, inflammatory and neoplastic changes. Lactobacillus *reuteri* have been revealed to decrease the gastric symptoms like diarrhea, inflammation, increase growth of pathogenic bacteria and decrease abdominal blockade which can cause disease like leaky gut syndrome (Rezaee *et al*., 2019). Lactobacillus *reuteri* has antimicrobial and immunomodulatory effect. Apart from killing the bacteria, Lactobacillus *reuteri* also kills the viruses like papillomavirus, rotavirus, circovirus and pneumovirus. It exhibits its antiviral effect by regulating the microbiota and releasing the metabolites which will kill the viruses. Lactobacillus *reuteri* also has antifungal effect, it helps in the killing of many

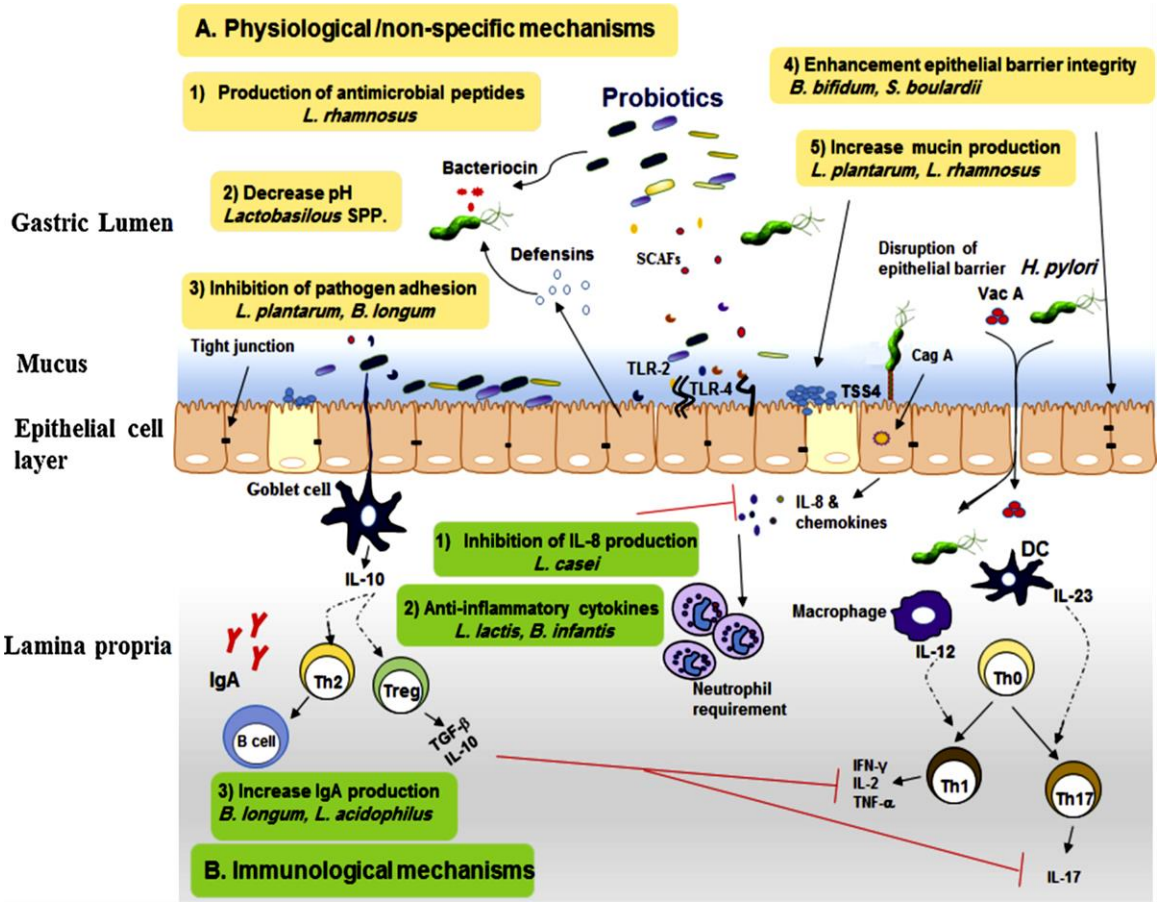


Figure 1.5: Possible functional mechanisms of probiotic bacteria for the treatment and elimination of *H. pylori* infection (Eslami, *et al*., 2019).

Candida species. It also inhibits histamine release which will generates anti-inflammatory effects (Mu *et al*., 2018). Lactobacillus *reuteri* also produces different types of essential vitamins like vitamin B12 and B9. There are four strains of *L. reuteri* which will help in vitamin production. For this reason, they can also be used for the treatment of disease which are caused due to vitamin B12 deficiency. *L. reuteri* also produces exopolysaccharide which will inhibit the adhesion of E. coli bacteria (Ksonzekova *et al*., 2016).

Lactobacillus *reuteri* also helps in the regulation of oral microbiota. It is induced into the subgingival mucosa to inhibit the development of periodontal disease (Mobini *et al*., 2017). It has also shown neuromodulatory effect. *L. reuteri* also prevents the visceral pain response especially by reducing the activity of enteric nervous system. *L. reuteri* also produces depressant effect by producing the gamma-aminobutyric acid (GABA), neurotransmitter (Lai *et al*., 2017). Lactobacillus *reuteri* also enhances the integrity of intestinal mucosal barrier and decreasing the penetrability of gastric mucosa by tight junction protein expression and by stopping apoptosis, so in this way it will prevent the development of leaky gut syndrome (Mu *et al*., 2018). The summary of these properties of Lactobacillus *reuteri* is shown in figure 1.6.

Rendering to Orel "Several Lactobacillus *reuteri* strains exhibit various characteristics such as secretion of antimicrobial reuterin, production of short-chain fatty acids, down-regulation of inflammatory immune response and direct influence on enteric nervous system among the others which render them good candidates for prevention and treatment of various FGIDs" (Rowles, 2017).

In a nutshell, the rational for using probiotics are they have shown to increase the abolition rates of *Helicobacter pylori* when used in combination with standard triple therapy by up to 10%. Patients who used probiotics apart from the standard triple therapy showed more improvement in their symptoms when compared to the pretreatment period. It is also noted that patients who received probiotics showed less side effects associated with standard regime (Hung *et al*., 2020).

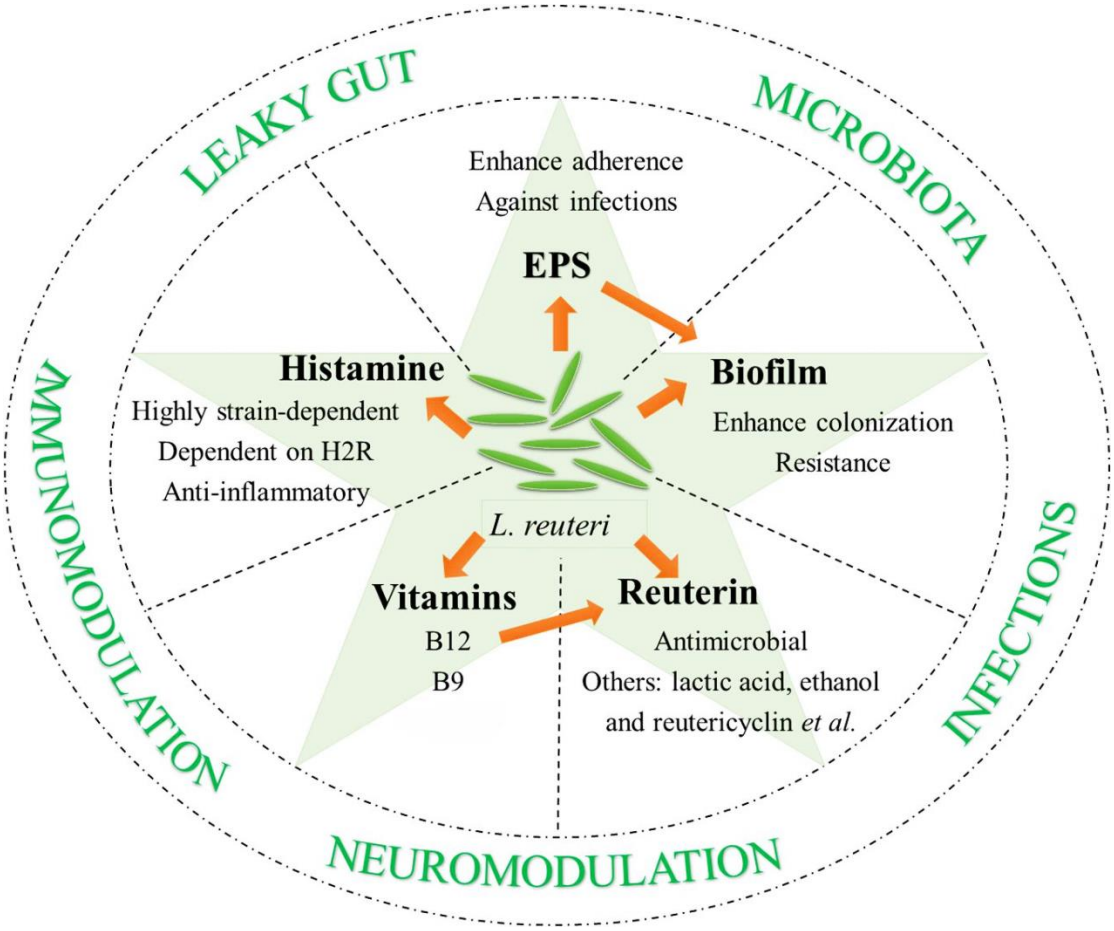


Figure 1.6: Probiotic properties of *L. reuteri* (Mu *et al.*, 2018).

1.2 HYPOTHESIS:

A) NULL HYPOTHESIS:

There is no difference in the effects of conventional triple therapy (Proton pump inhibitors + Clarithromycin and Amoxicillin) versus Lactobacillus *reuteri* + proton pump inhibitors for the treatment of peptic ulcer disease.

B) ALTERNATE HYPOTHESIS:

There is difference in the effects of conventional triple therapy (Proton pump inhibitors + Clarithromycin and Amoxicillin) versus Lactobacillus *reuteri* + proton pump inhibitors for the treatment of peptic ulcer disease.

1.3 OBJECTIVES OF STUDY:

This study will be conducted on Pakistani population having peptic ulcer disease with the following objectives:

1) To compare the efficacy of conventional triple therapy (Proton pump inhibitors + Clarithromycin and Amoxicillin) versus Lactobacillus *reuteri* + proton pump inhibitors in patients with peptic ulcer disease.

2) To compare the safety of conventional triple therapy (Proton pump inhibitors + Clarithromycin and Amoxicillin) versus Lactobacillus *reuteri* + proton pump inhibitors in patients with peptic ulcer disease.

1.4 STATEMENT OF THE PROBLEM:

At present, no therapy regime can guarantee 100% eradication of *H. pylori*. Antibiotic resistance of the *H. pylori* is the main cause of the failure of *H. pylori* treatment. Antibiotic therapy also has many severe adverse reactions like diarrhea, constipation, bloating, nausea, abdominal pain, liver function damage and fungal infection that can add to the agony of the patient and resulting in discontinuation of the treatment.

1.5 SIGNIFICANCE OF STUDY:

Peptic ulcer can occur at any age group but its frequency varies in the world population and can affect the people between the age of 30 to 60 years. Almost half of the world's population is colonized by *H. pylori*. It mainly colonizes in the human gastric antral mucosa and affects 95% of gastric and 70% of duodenal ulceration in the human. The mechanism by which *H. pylori* induces the development of different types of lesion in the stomach is not fully explained.

Helicobacter pylori is also the main cause of chronic active gastritis, dyspepsia, intestinal metaplasia, Mucosa-Association Lymphoid Tissue (MALT) lymphoma and gastric cancer. That shows the deleterious effect of *H. pylori* in the human body. That is why the eradication of *H. pylori* is very important.

The standard triple therapy includes proton pump inhibitor-clarithromycin and amoxicillin. But the recent data have shown that the efficacy of these regime has dropped to 70% successful eradication due to many reasons but the most important is resistance to clarithromycin. Due to these reasons, the exploration of new drug to treat *H. pylori* infection is need of the hour.

Probiotic like Lactobacillus *reuteri* is a newly discovered drug and have received increasing attention in recent years because of its safety. This dissertation will focus on the efficacy and safety of probiotic Lactobacillus *reuteri* + proton pump inhibitor combination in patients with peptic ulcer disease.

1.6 OPERATIONAL DEFINITIONS:

Peptic Ulcer Disease (PUD)

Peptic ulcer disease (PUD), also known as a peptic ulcer or stomach ulcer, is a break in the lining of the stomach, first part of the small intestine, or occasionally the lower esophagus. An ulcer in the stomach is known as a gastric ulcer while that in the first part of the intestines is known as a duodenal ulcer (Roy, 2016).

Gastroesophageal reflux disease (GERD)

Gastroesophageal reflux disease (GERD) is a clinical manifestation of the excessive reflux of acidic gastric contents into the esophagus causing various degree of symptomatic irritation or injury to the esophageal mucosa. Typical symptoms of GERD include heartburn, regurgitation and dysphagia (Gorecki, 2001).

Helicobacter pylori (H. pylori)

Helicobacter pylori (H. pylori) is a gram negative, spiral-shaped, microaerophilic human pathogen that colonizes the gastric mucosa of more than 50% of the global population. *H. pylori* is able to survive the acid environment of the stomach due to its ability to adhere to the gastric mucosa, colonizing the mucosal lining of the stomach (Muresan *et al.*, 2019).

Esophago-gastro-duodenoscopy

Esophagogastroduodenoscopy also called “upper endoscopy” is a medical procedure where a flexible lighted tube with a camera is inserted through the person’s mouth and into the stomach and duodenum to diagnose or treat disease. It is one of the most common procedures that a gastroenterologist performs (Ahlawat *et al.*, 2021).

Serologic antibody testing

Serologic antibody testing detects immunoglobulin G specific to *H. pylori* in serum, whole blood or urine and cannot distinguish between an active infection and a past infection. Most common serologic tests are based on enzyme-linked immunosorbent assay (ELISA) technology (Fashner & Gitu, 2015).

Probiotics

The Food and Agriculture Organization of the United Nations and the World Health Organization define probiotics as living microorganisms that are beneficial to life, can tolerate the effects of stomach acid, bile and pancreatic juice, can colonize the host’s gastrointestinal tract or reproductive system, induce host reactions and balance the intestinal flora to improve health (Song *et al.*, 2018).

CHAPTER 2

LITERATURE REVIEW

Stomach is a cylindrical J-shaped organ which lies beneath the lower part of the rib cage mainly in the left side of the hypochondrial region. It is an extended organ and has a capacity of around 1.5 litres. It has two openings one is proximal which connects with the esophagus and it is called cardiac orifice. Second is distal orifice which connects with the duodenum and it is called pyloric orifice. The stomach is divided into three parts 1. Fundus 2. Body 3. Pyloric region. The body is the largest and the broadest region of the stomach and it contains the parietal cells in the inner layer which produces hydrochloric acid. It receives blood supply from the coeliac artery (Mahadevan, 2017).

Stomach is a vital organ which helps in the digestion of food. It secretes gastrin from the G cells of gastric antrum and pepsin which helps in the digestion of proteins and also helps in the regulation of stomach acid secretion. Histamine is released from the ECL cells and is an important regulator of acid production. It binds to the H_2 receptor in the parietal cells stimulates adenylate cyclase which increases cyclic adenosine monophosphate (cAMP), resulting in dropping of gastric pH. Somatostatin inhibits the secretion of gastrin. Ghrelin, which is an important endocrine is released by ghrelinergic cells in the stomach. The gastric acid present in the stomach protects and kills the harmful microbes which are ingested into the body by food (Wu, 2019). Mucous cells are present throughout the stomach which serves as a protective barrier against harmful bacteria and from damaging by gastric acid. Prostaglandin acts as an important mediator of mucosal health. It activates the mucus and bicarbonate secretion and also prevents the gastric acid discharge by directly acting on parietal cells (Wilson & Stevenson, 2019).

Helicobacter pylori is the first microorganism which can persist in the harsh setting of the stomach. It is a gram-negative bacillus which is spiral-shaped. This microbe was first discovered by Marshall and Warren in 1983. These bacteria are more dominant in developing countries than developed nations. Approximately, 50% of the global population is affected by this pathogen. *H. pylori* is completely found in humans. It is transmitted mainly through close contact of one person to another via oral or other routes (Robinson *et al*., 2017). It produces infection mostly in people who belongs to low socio-economic status or who lives in crowded places and those who have poor hygiene. This bacterium has catalyzing and oxidizing activity. It uses glucose for phosphorylation and for energy and metabolism it requires amino acids like serine, histidine, leucine and valine (Ht, 2020).

The *H. pylori* can lead to a number to harmful diseases. There are two important virulence factors of *H. pylori* which plays an essential part in the growth of disease i.e. CagA and VacA. The most common disease which is produced by this pathogen is gastric and duodenal ulcers. It also produces functional dyspepsia, atrophic gastritis, gastric adenocarcinoma and gastric mucosal-associated lymphoid tissue (MALT) lymphoma (Roberts-Thomson, 2018). Several past studies have shown that the danger of peptic ulcer increases if anyone has previous history of *H. pylori* infection. For example, Cullen et al. described that peptic lesion is found more in patients who have *H. pylori*-induced gastritis as compared to those who do not have gastritis. If this pathogen is present chronically in the stomach it causes damage to the mucosal lining by damaging the delta cells and reducing the release of somatostatin. When this hormone is inhibited then automatically there will be increase production of gastric acid which will cause destruction of the gastric mucosa (Nejati *et al*., 2018).

The typical signs of peptic ulcer are abdominal pain which usually occurs at bedtime or at early morning and it is relieved after eating. Some other symptoms also occur like nausea, indigestion, cramps and heartburn (Altwejry *et al*., 2020). It should be diagnosed by taking proper history and by doing several tests. The diagnostic methods include urea breath test, serologic tests, fecal antigen test and confirmation of disease should be made by doing endoscopy. But endoscopy is an expensive and very time consuming process. It is also very uncomfortable procedure

for the patient. If biopsy is performed to diagnose *H. pylori*, then simple hematoxylin and eosin (HE) stain is used. A new histological technique can also be used to detect *H. pylori* i.e. Fluorescent in situ hybridization (FISH). It is also useful to detect other pathologies like gastric atrophy, gastritis and intestinal metaplasia. The polymerase chain reaction (PCR) is rarely used to identify *H. pylori* bacteria (Guevara & Cogdill, 2020).

To treat the *H. pylori* infection, the standard therapy was recommended that consists of two antibiotics which is typically clarithromycin and amoxicillin or metronidazole and a proton pump inhibitor which inhibits the secretion of stomach acid by acting on gastric acid pump i.e. (H+/K+ATPase). This therapy was used for a long time but due to increasing antibiotic resistance mainly to the clarithromycin and metronidazole the use of this standard therapy is unfortunately declining. Savoldi *et al*. demonstrated the worldwide antibiotic resistance (Mustafa, 2015). He found the incidence of clarithromycin resistance of 29% in European countries and 38% resistance to metronidazole. Similarly, in Africa the resistance to metronidazole was 75%, in Asia it was 46% and in USA it was 30%. There are many factors which leads to the antibiotic resistance. When there is mutation in bacteria it leads to decrease effectiveness of antimicrobial drug and an increase in bacterial burden also increases the resistance of microbes (Thaker *et al*., 2016).

According to the different geographic regions, first, second and even third line regimens have been developed and also many guidelines are made. According to the current guideline, the conventional triple therapy comprising of a Proton Pump Inhibitor and two antibiotics (clarithromycin/metronidazole and amoxicillin) which was previously discussed is considered the main line remedy for the annihilation of *H. pylori*. The time period of this is seven days in both Europe and Asia and ten to fourteen days in United States. But if anyone is allergic to amoxicillin or due to increase resistance to clarithromycin then metronidazole can be given as an alternative drug (Murata *et al*., 2020). According to a scientist Mabe *et al*. in area where resistance to clarithromycin is high then standard therapy which contains clarithromycin should not be used rather than metronidazole containing triple regimen should be used. Some studies have shown that both these therapies are effective but

some have also shown that the metronidazole-based triple therapy is more effective. Sun *et al*. discovered that metronidazole based therapy is good option for the treatment of peptic ulcer. According to one meta-analysis it is found that metronidazole containing triple therapy showed better efficacy than clarithromycin based regimen in persons of over 60 years of age diagnosed with peptic ulcer disease (Li *et al*., 2020). The efficacy of this triple therapy largely depends on PPI for example increasing the dose of this drug will automatically improve the success of triple therapy. This therapy should only be used in regions where the prevalence of clarithromycin tolerance is less than 15%. But in areas where the resistance rate is higher than 15% then bismuth containing quadruple remedy is considered as the main line therapy rendering to the Maastricht V/Florence consensus guideline. This regimen comprises of bismuth salicylate, proton pump inhibitor and two antibiotics (metronidazole and tetracycline) for 10-14 days. Several meta-analysis study of randomized controlled trials of bismuth quadruple therapy have shown that the 14-day course has higher abolition rate than the 7-day regimen (Lin TF *et al*., 2018).

A prospective, randomized, multicenter trial was conducted in North America in which comparison was done between Bismuth quadruple therapy (BQT) and Clarithromycin based triple treatment (CTT). In that trail, no noteworthy difference was found between these two therapies. However, another meta-analysis which was done in 2015 showed that BQT of 14 days was more effective than the 7 day's course of CTT (Guevara & Cogdill, 2020). Another study was done by Mantzaris *et al*. which compared the tolerability between BQT group and CTT group and demonstrated that the patients included in BQT group were more likely to experience side effects like nausea, headache and dizziness as compared to CTT group. According to one study it is revealed that when 14 day's bismuth quadruple therapy was combined with probiotics (L. *reuteri*) it showed higher cure rate and also reduces the side effects and increases the patient's compliance during treatment (Poonyam *et al*., 2019).

But due to the side effects produced by bismuth compound this therapy is no more used in many nations such as Japan, Malaysia and Australia. Alternative therapies like concomitant therapy or sequential therapy is used as main line therapies

in parts where there is high tolerance to clarithromycin. Concomitant therapy consists of three antibiotics i.e. clarithromycin, metronidazole and amoxicillin along with proton pump inhibitor for 10-14 days. This therapy was recommended by Toronto Consensus and Maastricht V/Florence Consensus (Yang *et al*., 2014).

A recent study was conducted in Greece where comparison between concomitant therapy and standard triple regimen was done. This study showed that the concomitant therapy had higher abolition rate of 90% than standard triple regimen that has 73.8% cure rate. Another meta-analysis was done in 2012 which evaluated 2070 patients divided between these two groups which again showed higher cure rate with concomitant therapy that is 90% versus 78% with standard therapy (Fakheri *et al*., 2014). A new clinical trial compared the concomitant treatment with sequential therapy and observed that there is no difference in the cure rate after completion of course. A second study between these two groups were done which included 164 patients who were identified with peptic ulcer disease. This study again showed the same results means there were no substantial difference found among the two groups with respect to eradication rate, compliance and side effects (Garza-González E *et al*., 2014).

Sequential therapy consists of dual therapy (PPI and amoxicillin) and triple therapy (PPI plus clarithromycin and metronidazole) for 5 days and has an eradication rate of 98%. This remedy was suggested by some Italian researchers in 2000. A meta-analysis of 10 randomized controlled trials was done to assess sequential therapy with conventional triple regimen. This analysis showed that the sequential treatment has higher abolition rate as compared to standard triple regimen. By this analysis it is concluded that sequential therapy can be used as a substitute to conventional triple regimen to cure the *H. pylori* infection. But rendering to the meta-analysis of 46 RCTs, in 2013 which was done by Gatta *et al*. it showed that the sequential regimen is only superior to standard therapy that is of 7 days only but not effective than 10 to 14 day's triple therapy. A new study was done which compared sequential treatment with concomitant regimen and discovered that the second therapy was better and safe than the first therapy (Kumar *et al*., 2020).

When both main-line therapy and bismuth quadruple regimen fails, a levofloxacin-based triple remedy is mentioned as rescue therapy. This treatment comprises of levofloxacin, amoxicillin and a proton pump inhibitor for 10 to 14 days. Also levofloxacin-amoxicillin quadruple therapy is also mentioned as second line treatment by the Maastricht V/Florence Consensus. This therapy is composed of levofloxacin, amoxicillin, PPI and bismuth compound for 10-14 days. A randomized controlled trial revealed that there was no difference between these two regimen for the annihilation of *H. pylori* (Rees *et al*., 2019). When this regimen was compared with clarithromycin based triple therapy (CTT) as a main line management then this regimen did not show any positive results but when this therapy was compared with clarithromycin based triple therapy (CTT) as a rescue treatment then levofloxacin-based triple remedy showed higher abolition rate than clarithromycin based triple regimen. From this study, it is concluded that levofloxacin-based triple remedy should only be used as second line regimen. A systemic review demonstrated that levofloxacin-amoxicillin triple remedy has a general cure rate of 78% as compared to concomitant therapy. But this therapy also has some drawbacks. Due to increase resistance of levofloxacin this therapy should be used carefully (Rothermel, 2020).

Hybrid therapy is a new treatment regimen and was first presented in 1989. It consists of proton pump inhibitor and amoxicillin for a duration of 7 days which is followed by a combination of three antibiotics (clarithromycin, metronidazole and amoxicillin) and proton pump inhibitor again for 7 days. According to recent guidelines this therapy is given in those areas where there is low resistance to clarithromycin. A study was done in Greece by Georgopoulos *et al.* which revealed that there is 50% decrease in the eradication rate in patients who lives in areas where there is tolerance to both clarithromycin and metronidazole drugs as compared to areas which is resistant to either clarithromycin or metronidazole. So, from this study we can conclude that hybrid therapy should not be given in regions where there is high resistance to these two antibiotics (Diaconu *et al*., 2017). A study was done which compared hybrid therapy and sequential therapy. This study showed that the first therapy has more cure rate that is 89.5% than the second therapy that has eradication rate of 76.7%. Another emerging second line therapy is high dose dual therapy. This therapy was first suggested in 1995 and has an abolition rate of more

than 90%. This therapy is composed of proton pump inhibitor and amoxicillin for a period of 14 days (DebanandaTudu and Jagadev 2020). A randomized control study was conducted in Taiwan in which comparison between fourteen days high dose dual therapy and sequential therapy was done. It showed that the high dose double treatment had higher cure rate (89%) than sequential therapy (52%) when used as a second-line therapy. A new study revealed that this treatment regimen had similar efficacy as levofloxacin-amoxicillin triple therapy of 7 days (79%). A randomized controlled trial was conducted in Germany that showed the high dose dual therapy had similar efficacy as that of bismuth quadruple remedy when used as a rescue treatment (Tosetti C *et al*., 2017).

Ellenrieder *et al.* in 1999 reported that 14-days dual therapy was effective as a salvage therapy as compared to 7-days triple therapy when used in patients who are resistant to clarithromycin and metronidazole providing strong evidence that this therapy should be used as a second-line regimen. Another scientist Sapmaz *et al* in 2017 conducted a study on 200 patients which revealed that the 14-days high dose double therapy had similar efficacy as that of 14-days bismuth-containing quadruple remedy and had an abolition rate of 84.7% and 87.8% respectively but it also revealed that the latter therapy had greater frequency of adverse effects like nausea, diarrhea, headache and stomach pain as compared to the first regimen (O'Connor et al., 2017). A similar result was found in the study conducted by Kim *et al* in 2012 which was done in 208 patients. In a recent study which was described by Yang J *et al.* in 2019 in 232 patients again showed the similar results that these two treatment regimen had comparable safety but 14-days high dose dual therapy was more economical than 14-day bismuth-containing quadruple therapy (Shamsdin *et al*.,2017). Another study was conducted by Miehlke *et al.* in 2003, which compared the 14-days high dose double therapy with bismuth-containing quadruple remedy as a rescue treatment, and demonstrated that both the therapies were effective and had an eradication rate of 75.6% and 81.4% respectively. In a comparable study which was conducted by Tai *et al.* in 2019 in which 240 *H. pylori* positive patients revealed that the 14-day high-dose double remedy with an eradication rate of 91.7% was more beneficial than the 7-day concomitant remedy and had a cure rate of 86.7% (Yun *et al.*, 2021).

A lot of the above mentioned studies revealed that the high-dose double therapy has a better eradication rate ranging from 84.7% to 95.3% and can be used as a first line treatment regimen and it can also be used as a rescue therapy having an abolition rate extending from 66.7% to 85.3%. It has an efficacy better than or equal to standard triple or quadruple therapies and also has less number of side effects and good patient compliance (Abbas *et al*., 2019). But some studies suggested that this therapy have less efficacy than other therapies. Like in a study which is presented by Hu *et al*. in 2017, it revealed that the fourteen-day high-dose double therapy was less effective than the bismuth-containing quadruple remedy and has an abolition rate of 81.6% and 84.3% respectively. Another study which was conducted by Graham *et al*. also showed the similar results (74.2%) (Gao *et al*., 2020).

Proton pump inhibitors is the most prescribed drug in every therapy whether it is first-line or second-line therapy to eliminate the *H. pylori* bacterium. It is also used to treat many diseases like gastro-esophageal reflux disease (GERD), inhibition of abdominal damage by non-steroidal anti-inflammatory drug acetylsalicylic acid (ASA), dyspepsia and peptic ulcer disease. It is also used to avoid the recurrence of NSAID-induced ulcers. Chronic use of NSAIDs causes mucosal damage and increase the susceptibility of *H. pylori* bacterium to cause local inflammation which further damages the gastric mucosa (Mössner 2016).

A meta-analysis of five studies was done by Vergara *et al*., in 2005 and assessed that by eradicating the *H. pylori* can NSAID-induced peptic ulcer be prevented and he found out that by annihilation of *H. pylori* the development of peptic lesion is significantly reduced. In another meta-analysis studies where comparison was done between *H. pylori* management versus proton pump inhibitor therapy, it is found that *H. pylori* elimination alone is less effective it should be done along with concomitant PPI administration for the treatment of NSAID-induced peptic lesion (Yadlapati and Kahrilas 2018). The Maastricht IV/ Florence Consensus Report mentions that annihilation of *H. pylori* is necessary in order to inhibit the development of NSAID-induced peptic lesion. But eradication alone will not fully treat the reoccurrence of peptic ulcers in patients who are chronic users of NSAIDs, in these high risk patients, prophylaxis by proton pump inhibitors should be done. If the

comparison is done between PPI and placebo it is found that this drug effectively reduces the risk of NSAID-induced peptic lesions. When literature search is done there is no evidence found that the increase dose of proton pump inhibitor will either enhance the healing or reduce the risk of gastric ulcers (Vaezi *et al.*, 2017). A scientist Yeomans *et al.* compared the omeprazole and ranitidine at 8 weeks and at 6 months, and demonstrated that omeprazole is more effective than ranitidine in preventing and healing the duodenal and gastric ulcers. When PPI is compared with misoprostol it is found that both the drugs had similar efficiency in the management of peptic ulcer associated with chronic use of NSAID. But according to the OMNIUM study it is found that proton pump inhibitors were more efficacious in reducing the duodenal ulcers but not effective in protecting the gastric ulcers as compared to misoprostol. From above studies it is concluded that the proton pump inhibitors are currently the drug of choice for preventing NSAID-related peptic ulcers (Melcarne *et al.*, 2016).

Management of *H. pylori* is very important because it can lead to gastric cancer. Several studies have shown that chronic use of proton pump inhibitors are also linked with gastric cancer. A study was done by Cheung *et al.* which showed a positive relation between PPI and stomach cancer in patients who are diagnosed with *H. pylori* (Joo *et al.*, 2019). Other disadvantages of chronic use of proton pump inhibitors are allergic reactions to chemicals like anaphylaxis, agranulocytosis, thrombocytopenia, hemolytic anemia. Patients may also complain of diarrhea and it also causes chronic nephritis. It also causes damage in the central nervous system such as dementia (Perry *et al.*, 2020). It may also cause pneumonia, increase the risk of gastric tumor, hypergastrinemia and it alters the composition of microbiota of gut. Long-term use reduces the absorption of calcium and vitamin B_{12} which leads to osteoporosis and increase the risk of bone fracture. Many studies have proposed the risk of femoral neck fracture in patients who are taking proton pump inhibitor drugs. It is also reported to cause hepatic encephalopathy in patients who have cirrhosis (Kinoshita *et al.*, 2018). When proton pump inhibitors like esomeprazole, rabeprazole and lansoprazole was compared with vonoprazan. It was found that vonoprazan is superior in efficacy and safety than proton pump inhibitors. Vonoprazan is a novel potassium- competitive acid inhibitor drug. A randomized double blinded study was done which demonstrated that vonoprazan based therapy when used as a main-line

and second-line remedies for the management of *H. pylori* it was found effective as compared to lansoprazole (Drini 2017). Sakurai *et al.* study revealed that vonoprazan decreases the secretion of acid much more quickly and obstinately as compared to the esomeprazole and rabeprazole. So, it is concluded that vonoprazan is beneficial than PPI-based therapy as first-line treatment but it also has more number of adverse effects than proton pump inhibitor. A new drug has shown to be effective in the management of stomach ulcer and due to its less number of side effects and decrease resistance it is getting a lot of attention (Sakurai K *et al.*, 2017).

Probiotics is a combination of Latin and Greek words which was first presented by German scientist Werner Kollath in 1953 “pro” means for and “biotic” means life. So it is simply a phrase of modern world meaning “for life”. The history of using probiotics dates back to the Egyptian era. Fermented milk was used in the Middle East mainly by the people of Egypt at the time of 10,000 BC, which is followed by the people of Greece and Italy. Also about 8000 BC, the Tibetan population used to have very good health because they daily consumed fermented yak milk. At the 11th century, the literature showed that Nomadic Turks used “yogurmak” to cure diarrhea, abdominal cramps and sunburned skin. Apart from that the great Mogul conqueror, Genghis Khan, used to fed yogurt to his huge army because it was reportedly believed that it inculcated courage in them. In 1905, a Russian scientist Elie Metchnikoff described probiotics as alteration in microflora present inside humans, and it will replace the damaging bacteria with good ones. But the major achievement came with the work of Henry Tissier, which detected that a specific type of bacteria was present in less concentration in the stool samples of children who were infected with diarrhea as compared to children who were healthy. He gave the idea of oral administration of live microorganism mainly Bifidobacteria to the patients who were infected and found to be helpful in the restoration of healthy gut flora was a novel effort (Liu *et al.,* 2018). A German scientist Alfred Nissle also gave the advice of using probiotic to the soldiers who were dying from the diarrheal infection in the World War 1. In the 1950s, more researches were done on probiotics like lactobacilli and bifidobacilli for the treatment of diarrheal infection and convincing results were found that the use of probiotics will prevent and treat diarrhea and also showed that it will assist in the healing of peptic ulcer. In 1974, Porker defined the probiotics as

"organisms and substances which contribute to the intestinal microbial balance" (Sanap *et al*., 2019).

According to WHO nutritional guidelines, probiotics can be defined as "live microorganisms when administered in adequate quantities confer a health profit to the host cell". Mostly species which are used as probiotics include bacteria and yeast. But bacterial species are most commonly used which belongs to Lactobacillus and Bifidobacterium genera. But yeast species like Enterococci, Propionibacteria and Saccharomyces have also been proposed (Dasari *et al*., 2017).

Lactobacillus belongs to the phylum *Firmicutes* and they are gram positive, rod-shaped, non-spore forming anaerobes which is used in the fermentation procedure of foods like milk, wine, fruits and vegetables and they are used for the preservation of foods like sauerkrauts, pickled vegetables, salami, olives and many more products. Currently, they are the most widely used bacteria for the food supplements and beverages. Bifidobacteria are also gram positive, anaerobic non-spore forming organisms. In the past few years, usage of Bifidobacterium as probiotic has markedly increased mainly in dairy products. But as compared to the Lactobacillus, it showed lesser features when used for the fermentation process (Linares *et al.,* 2017). Enterococci is a gram-positive cocci and it also belongs to *Firmicutes* phylum. They are lactic acid bacteria mostly found in the gut and bowel of human intestinal flora. Their species are isolated from milk, meat and fermented vegetables. They perform an important function of fermentation of cheese and production of taste, flavor and texture. Another probiotics *propionibacteria* also performs the same functions. *Saccharomyces* is a yeast probiotic which is also an important part of gut microflora. They have a key role in stimulation of defense system because they contain numerous immune-stimulants for example proteases, β-glucans and manna oligosaccharides (Gbadamosi *et al.,* 2020).

Probiotics are found in dairy and non-dairy products like in case of lactose intolerance, probiotics can be used to digest lactose by releasing the β-galactosidase enzyme in the small intestine. Probiotics are also found in fruits like grapes popularly called "hardaliye". It is a non-alcoholic beverage which is very famous in Turkey.

Like a peach probiotic named as L. *delbrueckii* was utilized for the fermentation of peach juice. Vegetables also have probiotic products. A very famous probiotic compounds is found in cabbage which is called “sauerkraut” which is used for the fermentation of many lactobacillus strains. Another compound kimchi is made by the use of garlic, onion, radish, cabbage and ginger (Yadav *et al.*, 2020). It contains a variety of health stimulating factors like it has anti-oxidative, anti-diabetic, anti-cancer, anti-obesity, anti-constipation and immune enhancing properties. That is why they are also used in the treatment of hypertension, diabetes and infections apart from the cure of diarrhea and peptic ulcer. Many researches had done on the anti-pathologic activities of probiotics which is considered as one of the most useful factor. Tejero-Sarinena *et al.* explored the impact of probiotic on the existence of some micro-organisms and found out that probiotic decreases the formation of pathogens by producing fatty acid short chains like acetic, butyric and lactic acids. Another scientist Islam also proposed that probiotic produce many kinds of anti-pathogenic compounds like ethanol, hydrogen peroxide, acetaldehyde and peptides (Chen *et al.*, 2019). Many studies had shown that probiotic inhibit the allergic reaction by lowering the inflammation, by improving the immune system of body, improving the health of microflora and most importantly by stabilizing the mechanism of healing to recover the damaged digestive system. Lactobacillus *GG* and L. *rhamnosus GG* are some of the probiotics which protect the body from allergic reactions. Probiotic also perform the function of lowering the blood pressure. It basically activates all the mechanisms which are involved in maintenance of blood pressure. Examples of these kinds of probiotics are Lactobacillus *casei*, Lactobacillus *rhamnosus, Bifidobacterium longum*, *Saccharomyces cerevisiae* and *Streptococcus thermophiles*. Probiotics had also shown to have positive effects in relieving symptoms of inflammatory bowel disease like diarrhea, stomach cramps and weight loss (Alvi *et al*., 2017).

About one billion females all over the world are suffering from urogenital infections which are not transmitted sexually like vaginitis and Urinary tract infection. The most abundant microorganism found in the vaginal duct are Lactobacillus species. When abnormal changes occur in the vagina it decreases the amount of Lactobacilli which results in the overgrowth of harmful bacteria (Ranjbar *et al.*, 2019). Some studies had also shown that probiotics have favorable effect in the

management of liver diseases. Large amount of normal microflora which are present in the stomach mucosa also have positive role in the proper functioning of liver cells and if any alterations occur in these microbes then it will lead to diseases like cirrhosis, hepatic encephalopathy and fatty liver disease. So probiotics improve the gut microflora and improve the immune system of body and also acts as a barrier by blocking the entry of harmful microbes in the blood stream (Javanmard A. *et al.,* 2018). Excessive amount of cholesterol in very bad for the health as it enhances the danger of coronary heart disease. Probiotics also performs a chief role in lowering the cholesterol level in the body. Like Lactobacillus probiotic are used to decrease the buildup of cholesterol in the adipocytes tissue. Probiotics also used to treat type 2 diabetes. It modifies the gut hormones such as gastric inhibitory polypeptide along with glucagon like peptides. Two scientist Prakash S & Lomis L did study the three different metabolically active strains of probiotic and found out that the combination of three strains were effective in the treatment of type 2 diabetes (Wang *et al.,* 2017). Various studies have shown that probiotics had positive effect on the brain and CNS also due to effects on the microflora of gut. In children who were suffering from autism probiotics like L. *plantarum* WCFS1 had shown improvement in those children. In those persons who were suffering from psychological distress Lactobacillus *helveticus* R0052 & R0175 had relieved the symptoms. In children who had symptoms of attention deficit syndrome probiotic such as L. *rhamnosus* might be helpful to reduce the symptoms. So, they were found to regulate the mood and behavior of people and also be given to reduce the depression (Sanap *et al*., 2019).

Probiotics could also be used to treat the respiratory tract diseases. Because they have several activities like anti-pathogenic action, anti-microbial action and immunomodulation so they might be helpful in curing the infections. Like strains of Lactobacillus *rhamnosus* and L. *plantarum* were used to treat nosocomial pneumonia. Probiotics can also be used to treat the oral diseases. Like they kill the harmful bacteria which are present in the oral cavity and protects the healthy gums and teeth. They inhibit the growth of microbes and adhere to the teeth surfaces and did not provide space for the bacteria to stick to the surfaces and cause dental caries (Jorgensen *et al*., 2017). Probiotics present in milk neutralizes the acid and those present in cheese stops the process of demineralization and stimulates the re-

mineralization process of enamel. They had also shown to reduce the number of S. mutants in dental plaque and also reduces periodontal diseases. Example of some probiotics are Lactobacillus *casei,* L. *reuteri,* L. *brevis*, L. *salivarius,* and *Bacillus subtilis* which would reduce the pathogens present in the periodontal tissues (Florea *et al*., 2020). They also had shown anti-cancer action. Many In-vitro studies had done and demonstrated that Lactobacillus had higher efficacy in depressing the cells of colorectal cancer and stimulates the growth of normal epithelial cells of colon by the formation of ferulic acid. Since probiotic activates the innate immunity and acts as an anti-pathogenic so it could also be used to treat the AIDS patient. It inhibits the replication of HIV virus and daily use of probiotic improve the amount of CD4 in the HIV virus (Chang *et al*., 2020). As previously studied, the probiotic helps in the annihilation of *H. pylori* bacteria. In a randomized, prospective controlled trail which is conducted by Ojetti *et al*. found out that when probiotic was added in the levofloxacin-based second-line therapy it increased the eradication rate to 20% as compared to the antibiotic therapy alone and also reduced the side effects of antibiotics (George Kerry R, *et al.,* 2018).

Probiotics have also been used to treat ulcers which are caused by frequent use of NSAIDs such as aspirin and indomethacin. It also helps in the treatment of lupus erythematosus by improving renal function and reducing serum autoantibodies. It also maintains the body weight by increasing the activity of gastric microbiota. Probiotics also have many anticancer properties. It helps in the prevention of colorectal carcinoma which is the third most common cancer worldwide, gastric carcinoma, liver cancer which is the second most common cause of death globally. They are also used to prevent the cancer complications. In addition to these advantages, probiotics have also shown to be effective against metabolic diseases like hyperlipidemia, obesity and diabetes. Therefore, it reduces the risk of atherosclerosis and hypertension (Emara *et al.,* 2016).

Armuzzi *et al*. did experiment on 120 patients who were asymptomatic and Hp positive and divided them into two categories. One group was given triple therapy and other group was given combination of triple therapy along with probiotic Lactobacillus bid for 14 days. He concluded that the group which received probiotic

along with triple therapy showed significant improvement in gastric symptoms like bloating, diarrhea and taste but no difference were found in the eradication rate. Chitapanarux *et al.* evaluated the effectiveness of probiotic Bifidobacterium in a double-blinded, placebo-controlled study. He combined B. longum with standard triple therapy he found out that it had better eradication rate and produced less number of side effects. Another scientist Song *et al.* added the Saccharomyces boulardii, which is a yeast probiotic to the first-line therapy and after 4 weeks concluded that the eradication rate increased to 85.4% as compared to 80% when probiotics were not added (Castro-González *et al*., 2019).

A meta-analysis of ten studies were carried out by Wang *et al.* which revealed that probiotics compounds like Lactobacillus and Bifidobacterium species enhances the abolition rate of *H. pylori* and also decreases the harmful effects associated with antibacterial therapy. Another scientist Mcfarland *et al.* also done meta-analysis and confirmed that many strains of probiotics could be helpful but not all the combination of strains were equally effective. Lastly, Lau *et al.* concluded that the strains of Lactobacillus, Bifidobacterium, Saccharomyces and other mixtures should be used for the cure of *H. pylori* disease in both children and adults living in Asian and non-Asian population (Bruno *et al.,* 2018).

CHAPTER 3

METHODOLOGY

3.1 Study design:

Randomized clinical trial (Prospective).

3.2 Subjects / Animals:

Males and females ≥ 18 years of age fulfilling the inclusion criteria were recruited in the study after informed consent.

3.3 Place of sample collection / Setting:

Medical OPD of National Medical Centre, Karachi

3.4 Inclusion criteria:

Patients having following features will be included in the study:

1. Male and female of age 18 to 50 years.

2. Epigastric pain 6 weeks or more duration.

3. *H. pylori* stool antigen assay test positive.

3.5 Exclusion criteria:

- Male and female of age less than 18.
- Patient with a history of cancer.
- Patient with a history of co-morbid diseases.
- Patient who are regular user of non-steroidal anti-inflammatory drugs or bisphosphonates, oral intake of antibiotics or PPIs in the precedent 3 months.
- Negative stool antigen test for *H. pylori*.
- Pregnancy or lactation.

3.6 Duration of study:

(a) Individual study period

2 Weeks

(b) Total period of study

6 months (October 2020- March 2021)

3.7 Sample size estimation:

Sample Size For Comparing Two Means

Input Data

Confidence Interval (2-sided)	95%		
Power	80%		
Ratio of sample size (Group 2/Group 1)	1		
	Group 1	**Group 2**	**Difference***
Mean	5.25	4.65	0.6
Standard deviation	0.86	0.93	
Variance	0.7396	0.8649	
Sample size of Group 1	35		
Sample size of Group 2	35		
Total sample size	70		

*Difference between the means (Dore *et al.*, 2019)

Results from OpenEpi, Version 3, open source calculator—SSMean

$n = (Z_{\alpha/2}+Z_{\beta})^2 * 2*\sigma^2 / d^2$,

where $Z_{\alpha/2}$ is the critical value of the Normal distribution at $\alpha/2$ (e.g. for a confidence level of 95%, α is 0.05 and the critical value is 1.96), Z_{β} is the critical value of the Normal distribution at β (e.g. for a power of 80%, β is 0.2 and the critical value is 0.84), σ^2 is the population variance, and d is the difference you would like to detect.

The calculated sample size was 70 with 35 patients in each group. However, we have enrolled 100 patients with 50 patients in each group.

3.8 Sampling technique:

Systemic random sampling (randomized sampling technique)

3.9 Human subjects:

100 individuals

3.10 Materials used (Drugs/ Chemicals/ Proforma /Questionnaire/any other):

Drugs:

(1) Triple therapy (Omeprazole 20 mg BD + Clarithromycin 500 mg BD + Amoxicillin 1 gm BD) (Leow *et al*., 2020)
(2) Cap Lactobacillus *reuteri* 100 mg BD (Dore *et al*., 2019)

Instruments:

(1) Spectrophotometer
(2) ELISA Reader
(3) Immunoassay analyzer
(4) Automated chemistry analyzer
(5) Hematology analyzer
(6) Refrigerator
(7) Freezer

Kits:

(1) Stool antigen testing kit (Company Healgen)
(2) LFTs Kit (Company Merck)

Other;

(1) Subject evaluation form

3.11 Parameters of study:

1) Stool antigen assay
2) Complete blood count
3) Liver function test
4) Renal function test

3.12 Protocol of study:

After the ethical approval of clinical trial from ethical review committee of Bahria university (BUMDC) and National Medical Centre (NMC).

Patients fulfilling the inclusion criteria were enrolled for the study after taking informed written consent (attached).

All required information such as name, age, sex, occupation, address, phone contact, associated illness including trauma, hepatic, renal, cardiovascular etc., were recorded on prescribed Performa specially designed for the study.

A wash out period of at least 72 hours for any medication to the patient were given before the start of the study. 100 diagnosed peptic ulcer disease patients with positive stool antigen test for *H. pylori* were selected from Medical OPD of National Medical Centre Karachi. The patients were divided randomly into two groups, each consisting of 50 patients.

Group A:

50 patients with positive stool antigen test were enrolled with triple therapy (Proton pump inhibitor 20 mg BD + Clarithromycin 500 mg BD + Amoxicillin 1 gm BD) for 14 days and then again followed by stool antigen assay.

Group B:

50 patients with positive stool antigen test were enrolled with Lactobacillus *reuteri* (Cap Lactobacillus *reuteri* 100 mg BD + proton pump inhibitors 20 mg BD) for 14 days and then again followed by stool antigen assay.

Base- line investigations:

Before enrolment for the study necessary investigations was done:

1) Complete blood count
2) Stool antigen assay
3) Liver function test
4) Renal function test

All the parameters mentioned above were recorded on 0 and 14 days.

3.12.1 Complete Blood Count (CBC) (Doig & Zhang, 2017)

This method is a set of all laboratory tests which is performed to get complete information about the cells present in a human's blood. The CBC shows the count of white blood cells, red blood cells and platelets, concentration of hemoglobin and the hematocrit.

Principle:

This test is performed by using Sysmex XN 550 hematology analyzer. This device is a multi-parameter quantitative automated hematology analyzer which is used for determination of whole blood parameters. Sysmex XN 550 detects hemoglobin by the cyanide free sodium lauryl sulphate (SLS) method. The red blood cells and white blood cells are lysed by the regent sulfolyser present in the sample. It starts working

by altering the globin chain and then oxidizing the heme group. After this SLS, the hydrophilic groups bind to the heme group and forms the colored complex (SLS-HGB). It is a stable compound which is analyzed by using spectrophotometer. An LED sends monochromatic light and when it passes through the mixture it is absorbed by SLS-HGB complexes. The absorbance of the resulting color is proportional to the concentration of hemoglobin.

Required specimen**:** Whole blood anticoagulated with a potassium EDTA is preferred.

Reagent storage: Stored at 1^{o} -30^{o} C away from direct sunlight.

Reference value:

Hemoglobin (Hb): Adult male: 135-180 g/L

Adult female: 120-160 g/L

White blood cells (WBCs): 4,500-11,000/µL

Platelets: 150,000 to 450,000 µ/L

3.12.2 Stool Antigen Assay (Pronovost *et al*., 1994)

This test is used to detect *H. pylori* antigen in feces. It is a lateral flow chromatographic immunoassay which is only used for in vitro diagnosis.

Principle:

The stool antigen test is based on the principle of double antibody-sandwich technique. This test kit consists of:

1) A burgundy colored conjugate pad containing *H. pylori* antibodies conjugated with color particles.

2) A nitrocellulose membrane strip containing a test band (T band) and a control band (C band). The T band is pre-coated with non-conjugated *H. pylori* antibodies.

When an adequate volume of test specimen is dispensed into the sample well of the cassette, the specimen migrates by capillary action across the cassette. The antigen of *H. pylori* if present in the specimen will bind to the *H. pylori* antibodies conjugates. The immunocomplex is then captured on the membrane by the pre-coated *H. pylori* antibodies, forming a burgundy colored T band, indicating a *H. pylori* antigen positive test results.

Required specimen: sufficient quantity of feces (1-2 ml or 1-2 g).

Storage: All reagents should be stored at 2^{o} C- 30^{o} C.

Specimen Collection:

Collect sufficient quantity of feces (1-2 ml or 1-2 g) in a clean, dry specimen collection container to obtain maximum antigens (if present). Best results will be obtained if the assay is performed within 6 hours after collection. Specimen collected may be stored for 3 days at 2-8 ^{o} C if not tested within 6 hours. For long term storage, specimens should be kept below -20 ^{o} C.

To process fecal specimens:

1. **For Solid Specimens:**

Unscrew the cap of the specimen collection tube, then randomly stab the specimen collection applicator into the fecal specimen in at least 3 different sites to collect approximately 50 mg of feces (equivalent to 1/4 of a pea). Do not scoop the fecal specimen.

2. **For Liquid Specimens:**

Hold the dropper vertically, aspirate fecal specimens, and then transfer 2 drops (approximately 80 µL) into the specimen collection tube containing the dilution buffer. Screw on and tighten the cap onto the specimen collection tube, then shake the specimen collection tube vigorously to mix the specimen and the dilution buffer. Leave the tube alone for 2 minutes.

Procedure:

1) After collecting the sample, hold the sample collection device upright and carefully break the tip of the collection device.
2) Now squeeze 2 drops of the sample solution in the sample well of the cassette and read the test results in 10 minutes. Do not read the results after 10 minutes.

Interpretation of results:

If the test result is positive, then two lines appear. One colored line should be in the control line region (C) and another apparent colored line should be in the test line region (T).

If the test result is negative, then only one colored line appears in the control line region (C). No line appears in the test line region (T).

3.12.3 Liver Function Test (LFT):

3.12.3.1 Aspartate transaminase (AST) (Kaplan *et al*., 2003)

Principle:

AST in the sample catalyzes the transfer of an amino group between L-aspartate and 2-oxyglutarate to form oxaloacetate and L-glutamate. The oxaloacetate reacts in the next chemical reaction with NADH, in the presence of malate dehydrogenase (MDH) to form NAD^+. The rate of NADH oxidation is directly proportional to the catalytic AST activity. It is measured photometrically by decrease in the absorbance.

Sample: Plasma

Storage and stability: Reagents were stored at +2 to +8˚C

Procedure notes:

The results were reported to the nearest whole number in U/L.

Analytical Measurement Range (AMR): 3 U/L - 1200 U/L. In the decrease mode, the extended measuring range with the automatic rerun function (1:11) of the analyzer was 3-13200 U/L. The results from the diluted sample, utilizing the rerun function, were automatically multiplied by a factor of 11.

Values less than 3 were reported as <3 U/L.
Values more than 13200 were reported as >13,200 U/L.

Calculations:
The COBAS 6000/8000 system was automatically calculated the GGT activity of each patient sample.

Reference value:

Male: 8-61 U/L

Female: 5-36 U/L

3.12.3.2 Alanine aminotransferase (ALT) (Henderson & Moss, 2001)

Principle:

In the presence of α-ketoglutarate, alanine is transformed into pyruvate and glutamate by ALT/GPT in the sample. In presence of NADH and lactate dehydrogenase, pyruvate is converted into lactate and NAD.

α-Ketoglutarate + L-Alanine ↔ L-Glutamate + Pyruvate

Pyruvate + NADH + H ↔ L-Lactate + NAD^+

Sample: Serum, plasma with heparin or EDTA.

Storage: Reagents were stored at +2 to +8˚C.

Procedure:

Serum or plasma: 50 µl

Reagent solution: 500 µl

Mix after 1 minute add start reagent 125 µl. Mix after approx. 1minute measure the decrease in absorption every minute for 3 minutes.

Expected values:

Male: ≤ 40 U/L

Female: ≤ 32 U/L

3.12.4 Renal Function Test:

3.12.4.1 Serum Urea (Gounden *et al*., 2021)

Principle:

Urea in the sample is hydrolyzed enzymatically into ammonia (NH_4^+) and carbon dioxide (CO_2). Ammonia ions formed reacts with α-ketoglutarate in a reaction catalyzed by glutamate dehydrogenase (GLDH) with simultaneous oxidation of NADH to NAD^+:

$$\text{Urea} + H_2O + 2\,H^+ \xrightarrow{\text{Urease}} (NH_4^+)_2 + CO_2$$

$$NH_4 + \alpha\text{-ketoglutarate} + \text{NADH} \xrightarrow{\text{GLDH}} H_2O + NAD^+ + \text{L-Glutamate}$$

The decrease in concentration of NADH, is proportional to urea concentration in the sample.

Sample: Serum or heparinized plasma

Storage: Urea should be stored at 2-8 ^{o}C.

Procedure:

1) Assay conditions:

Wavelength: 340 nm

Cuvette: 1 cm light path

Temperature: 37^{o} C / 15-25 ^{o}C

2) Adjust the instrument to zero with distilled water.
3) Pipette into a cuvette.
4) Mix and read the absorbance after 30s (A1) and 90s (A2).
5) Calculate: $\Delta A = A1 - A2$

Reference range:

Serum or plasma: 15 – 45 mg/dl = 2.5 – 7.5 mmol/L

3.12.4.2 Serum Creatinine (Gounden *et al*., 2021)

Principle:

Creatinine forms a Yellow-orange compound in alkaline solution with Picric acid. At the low picric acid concentration used in this method a prescription of protein does not take place. The concentration of the dyestuff formed over a certain reaction times is a measure of the creatinine concentration.

As a result of the rapid reaction between creatinine and picric acid, later secondary reactions do not cause interference.

Sample: Serum, heparin plasma, urine.

Storage: Stored at +2 to +8 ^{o}C for 24 hours.

Procedure:

Since the reaction is highly temperature-sensitive, care must be taken to ensure that the solutions are preheated to an exact temperature and the reaction must proceed at a constant temperature. The reaction temperature of the standard and that of the sample must be identical. Provided these conditions are fulfilled, the determination can be performed at any chosen temperature between +20 and 37 ^{o}C.

Measure the absorbance of the sample (A_S) and standard (A_{St}) after 1 min. Exactly 2 min after the first measurement.

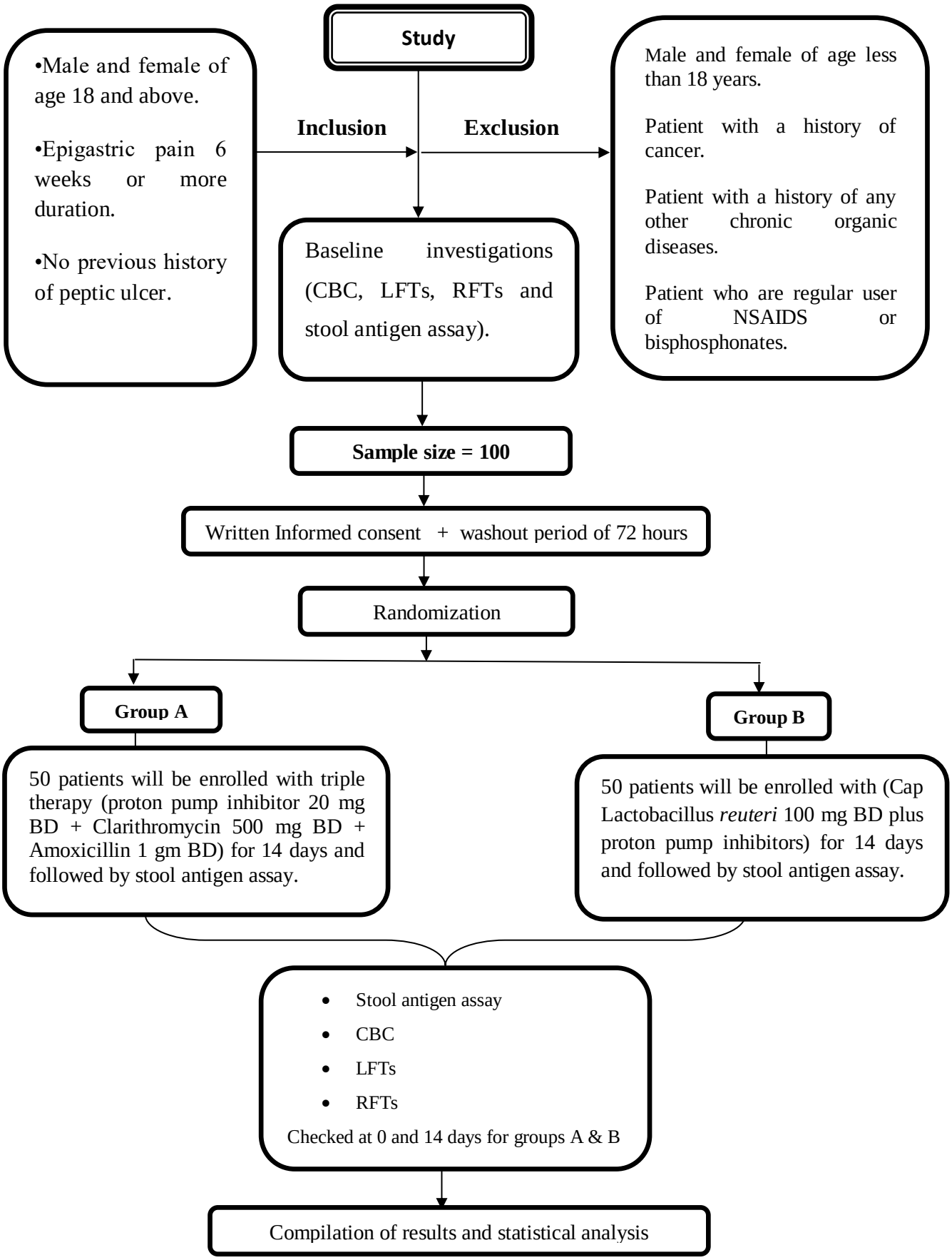
Redetermine the absorbance of the sample (A_S) and the standard (A_{St}). If the reaction is performed at a temperature above +37 ^{o}C read absorbance A_1 after 30 seconds.

Normal range:

Female: 0.6 – 0.9 mg/dl (53 – 80 µmol/l)

Male: 0.7 – 1.1 mg/dl (62 – 97 µmol/l)

3.13 Algorithm of study:



3.14 Statistical analysis:

The data of the patients were analyzed by using the IBM statistical package of social sciences (SPSS) software version 23.0. The results were expressed as mean ± standard deviation (SD). The qualitative variables were shown in frequency and percentages. To find out the difference between Pre and Post findings, independent T-test and Paired T-test were used. For qualitative variables Chi-square test was applied. P-value < 0.05 was considered as statistically significant.

CHAPTER 4

RESULTS

Total 100 patients of either gender were enrolled in this study. 92 patients completed the study with 46 patients in each group. Male and female between the age of 18 years to 50 years fulfilling the inclusion criteria were included in the study after taking the written informed consent to evaluate and compare the efficacy and safety of conventional triple therapy (Proton pump inhibitors + Clarithromycin and Amoxicillin) versus Lactobacillus reuteri + proton pump inhibitors in patients with peptic ulcer disease.

In both study groups, Group A conventional triple therapy (Proton pump inhibitor + Clarithromycin + Amoxicillin) and group B (Lactobacillus reuteri + proton pump inhibitors) 50 patients were included but 4 patients left out during the study from each group. Descriptive statistics were calculated using SPSS version 23. Qualitative variables were presented in terms of frequency and percentage. Quantitative variables were presented in term of mean and standard deviations. Mean comparison was done by independent t-test and qualitative variables were tested by using Chi-square test. The p value ≤ 0.05 was considered as statistically significant.

4.1 Hemoglobin (Hb) (Group A & B):

Mean Hb in group A at baseline and after 14 days was found to be same i.e. 15.00±1.321 % while mean Hb in group B at baseline and after 14 days was also same i.e. 14.94±1.399 %. We found non-significant mean difference for Hb in group A at

baseline and after 14 days (p=0.999). We found non-significant mean difference for Hb in group B at baseline and after 14 days (p=0.999). When we compared both the groups, group A and group B we also found non-significant mean difference at baseline and after 14 days (p=0.741) (Table 4.1, Table 4.2, Table 4.3, Figure 4.1, Figure 4.2 and Figure 4.3).

Table 4.1: Hb (Hemoglobin), Group A, Day 0 v/s Day 14, (N= 46)

	N	Mean	Std. Deviation	Std. Error Mean	P-Value
Baseline	46	15.00	1.321	.197	0.999^{NS}
After 14 days	46	15.00	1.321	.197	

Group A: treated with conventional triple therapy (Proton pump inhibitor + Clarithromycin + Amoxicillin), P value < 0.05: significant, *: Statistically significant, NS=Not Significant at 0.05. Test applied: Independent t-test.

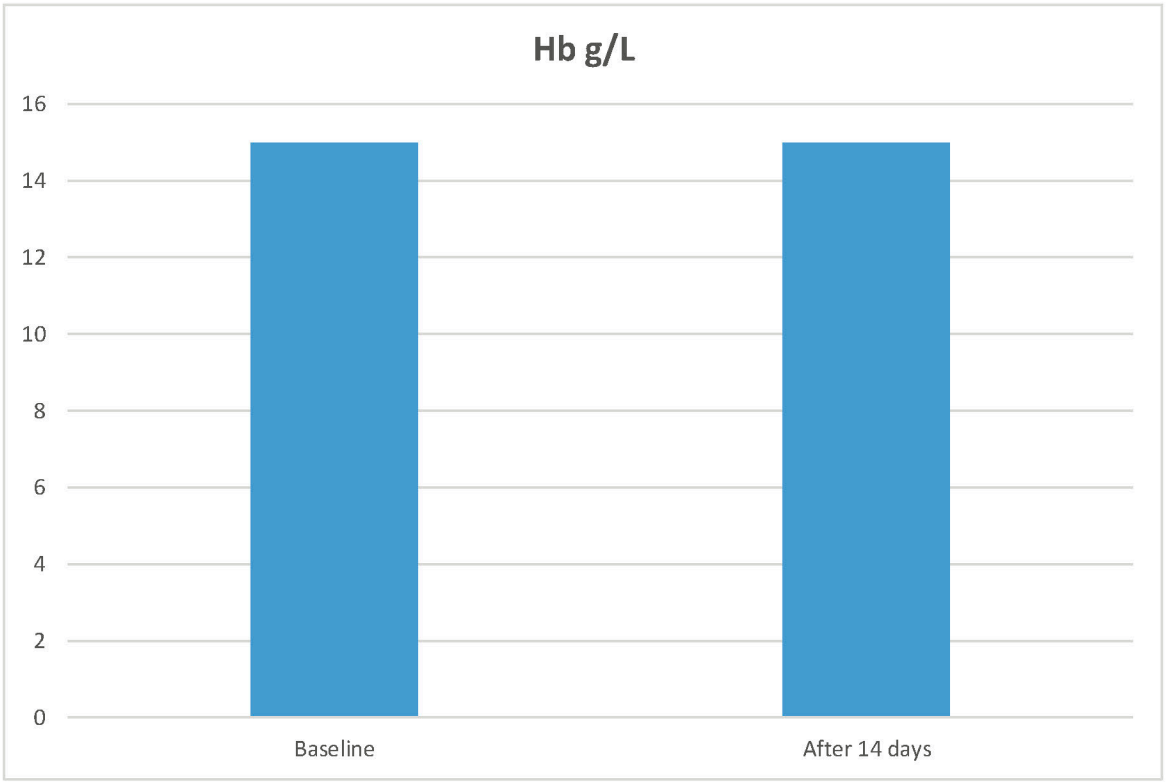


Figure 4.1: Hb (Hemoglobin), Group A, Day 0 v/s Day 14, (N= 46)

Table 4.2: Hb (Hemoglobin), Group B, Day 0 v/s Day 14, (N= 46)

	N	Mean	Std. Deviation	Std. Error Mean	P-Value
Baseline	46	14.94	1.399	.209	0.999^{NS}
After 14 days	46	14.94	1.399	.209	

Group B: treated with combination therapy (Lactobacillus reuteri + proton pump inhibitors), P value < 0.05: significant, *: Statistically significant, NS=Not Significant at 0.05. Test applied: Independent t-test.

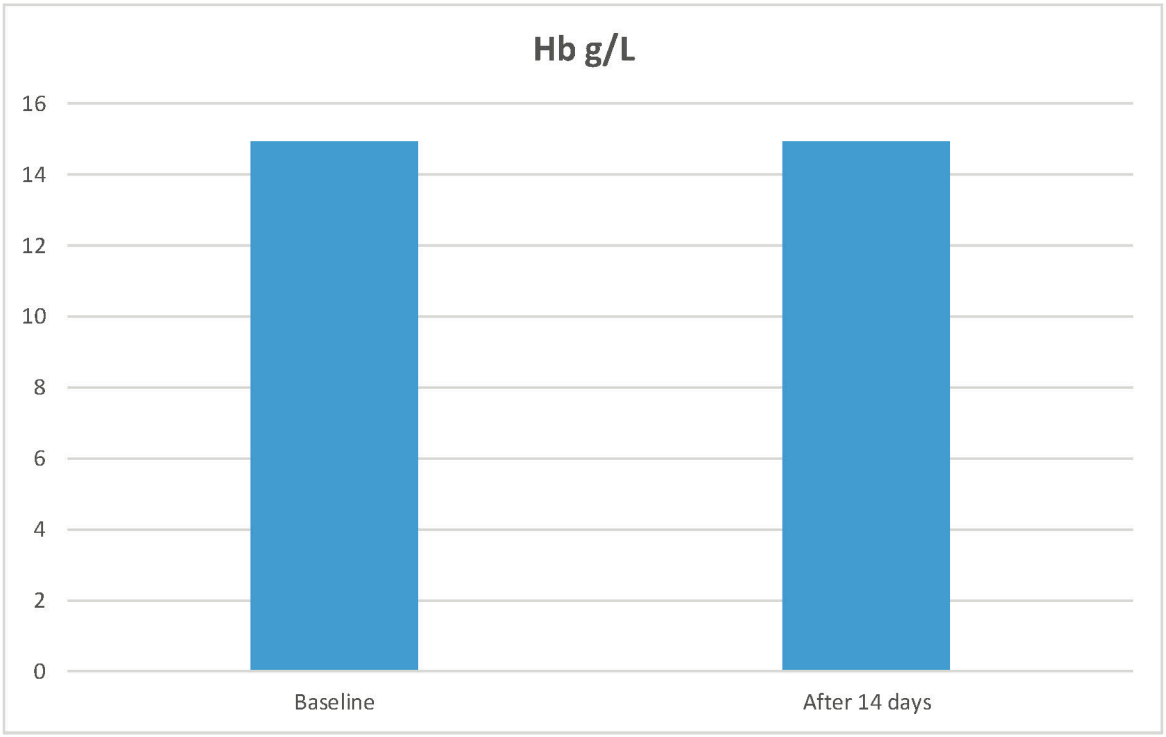


Figure 4.2: Hb (Hemoglobin), Group B, Day 0 v/s Day 14, (N= 46)

Table 4.3: Hb (Hemoglobin), between group A and group B (N= 92)

	N	Mean	Std. Deviation	Std. Error Mean	P-Value
GROUP A	46	15.00	1.313	.138	0.741^{NS}
GROUP B	46	14.94	1.391	.147	

Group A: treated with conventional triple therapy (Proton pump inhibitor + Clarithromycin + Amoxicillin); Group B: treated with combination therapy (Lactobacillus reuteri + proton pump inhibitors), P value < 0.05: significant, *: Statistically significant, NS=Not Significant at 0.05. Test applied: Independent t-test.

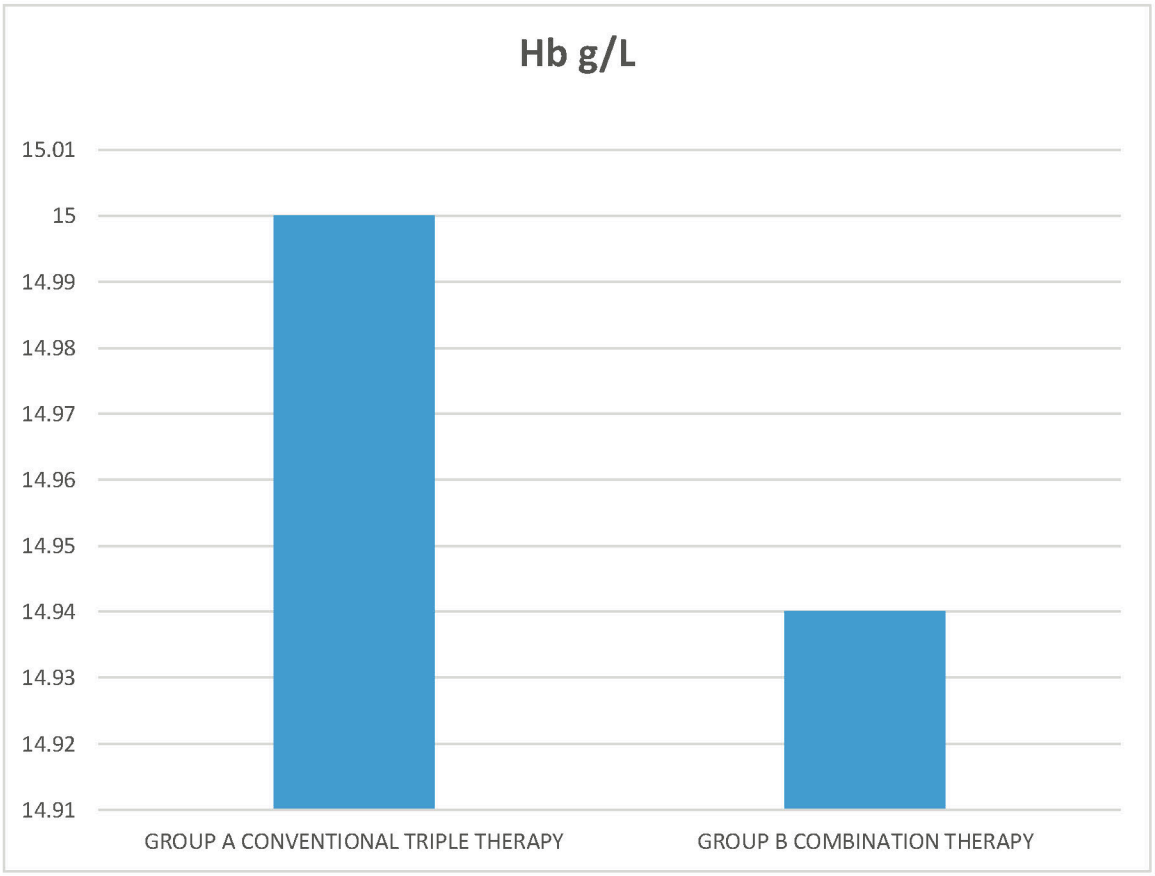


Figure 4.3: Hb (Hemoglobin), between group A and group B (N= 92)

4.2 Liver Function Test (Group A & B):

4.2.1 Alanine aminotransferase (ALT):

Mean ALT in group A at baseline and after 14 days was found to be same i.e. 25.67±4.033 IU/L while mean ALT in group B at baseline and after 14 days was also same i.e. 25.46±4.037 IU/L respectively. We found non-significant mean difference for ALT in group A at baseline and after 14 days (p=0.999). We found non-significant mean difference for ALT in group B at baseline and after 14 days (p=0.999). When we compared both the groups, group A and group B we also found non-significant mean difference at baseline and after 14 days (p=0.714) (Table 4.4, Table 4.5, Table 4.6, Figure 4.4, Figure 4.5 and Figure 4.6).

4.2.2 Aspartate transaminase (AST)

Mean AST in group A at baseline and after 14 days was found to be same i.e. 28.83±5.335 units/L while mean AST in group B at baseline and after 14 days was also same i.e. 29.59±4.440 units/L respectively. We found non-significant mean difference for AST in group A at baseline and after 14 days (p=0.999). We found non-significant mean difference for AST in group B at baseline and after 14 days (p=0.999). When we compared both the groups, group A and group B we also found non-significant mean difference at baseline and after 14 days (p=0.292) (Table 4.7, Table 4.8, Table 4.9, Figure 4.7, Figure 4.8 and Figure 4.9).

Table 4.7: ALT (IU/L), Group A, Day 0 v/s Day 14, (N= 46)

	N	Mean	Std. Deviation	Std. Error Mean	P-Value
Baseline	46	25.67	4.033	.595	0.999^{NS}
After 14 days	46	25.67	4.033	.595	

Group A: treated with conventional triple therapy (Proton pump inhibitor + Clarithromycin + Amoxicillin), P value < 0.05: significant, *: Statistically significant, NS=Not Significant at 0.05. Test applied: Independent t-test.

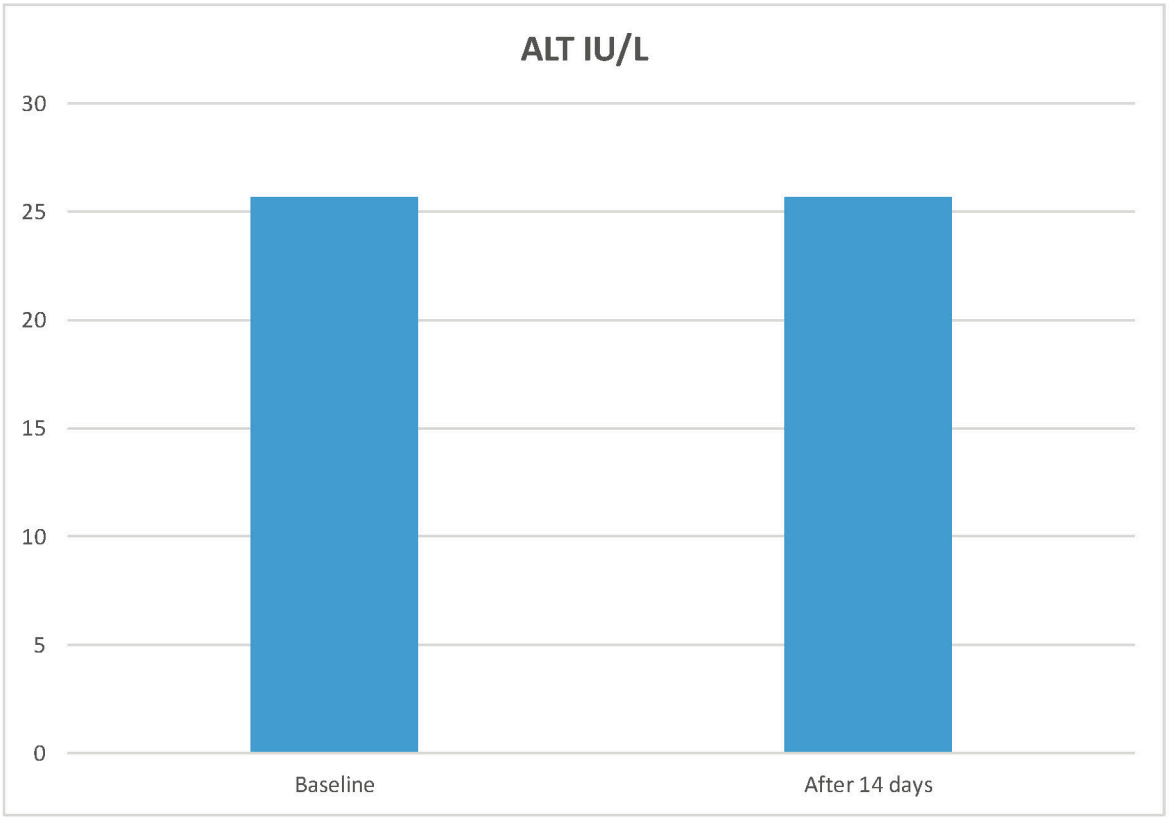


Figure 4.7: ALT (IU/L), Group A, Day 0 v/s Day 14, (N= 46)

Table 4.8: ALT (IU/L), Group B, Day 0 v/s Day 14, (N= 46)

	N	Mean	Std. Deviation	Std. Error Mean	P-Value
Baseline	46	25.46	4.037	.595	0.999^{NS}
After 14 days	46	25.46	4.037	.595	

Group B: treated with combination therapy (Lactobacillus reuteri + proton pump inhibitors), P value < 0.05: significant, *: Statistically significant, NS=Not Significant at 0.05. Test applied: Independent t-test.

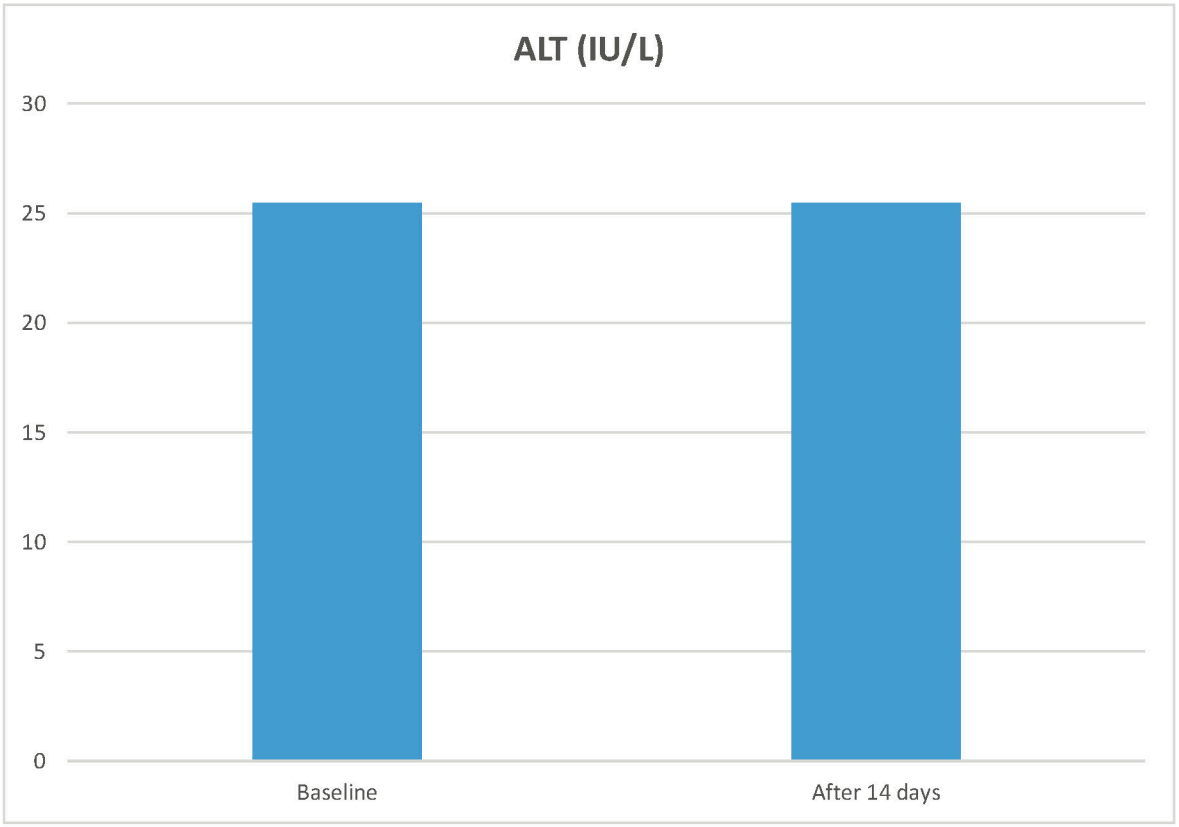


Figure 4.8: ALT (IU/L), Group B, Day 0 v/s Day 14, (N= 46)

Table 4.9: ALT (IU/L), between group A and group B (N= 92)

	N	Mean	Std. Deviation	Std. Error Mean	P-Value
GROUP A	46	25.67	4.011	.418	0.714^{NS}
GROUP B	46	25.46	4.015	.419	

Group A: treated with conventional triple therapy (Proton pump inhibitor + Clarithromycin + Amoxicillin); Group B: treated with combination therapy (Lactobacillus reuteri + proton pump inhibitors), P value < 0.05: significant, *: Statistically significant, NS=Not Significant at 0.05. Test applied: Independent t-test.

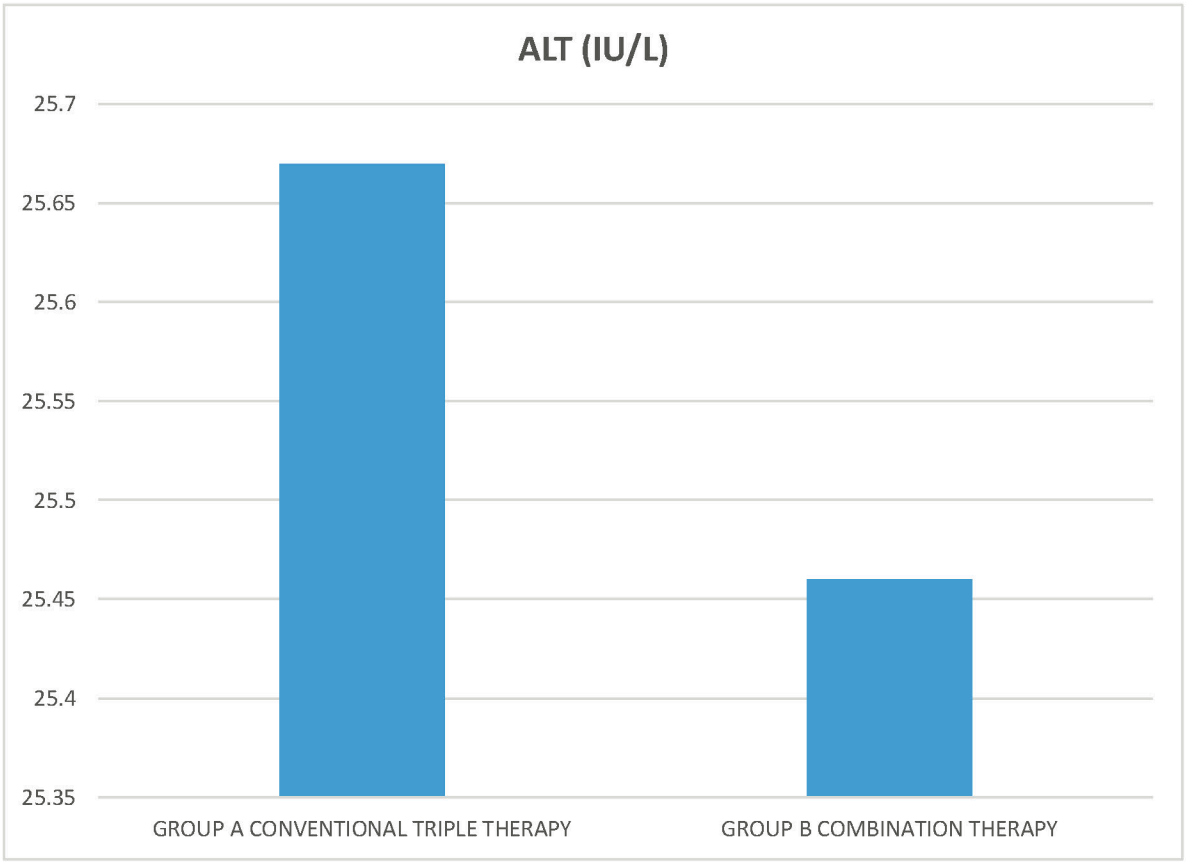


Figure 4.9: ALT (IU/L), between group A and group B (N= 92)

Table 4.10: AST (units/L), Group A, Day 0 v/s Day 14, (N= 46)

	N	Mean	Std. Deviation	Std. Error Mean	P-Value
Baseline	46	28.83	5.335	.787	0.999^{NS}
After 14 days	46	28.83	5.335	.787	

Group A: treated with conventional triple therapy (Proton pump inhibitor + Clarithromycin + Amoxicillin), P value < 0.05: significant, *: Statistically significant, NS=Not Significant at 0.05. Test applied: Independent t-test.

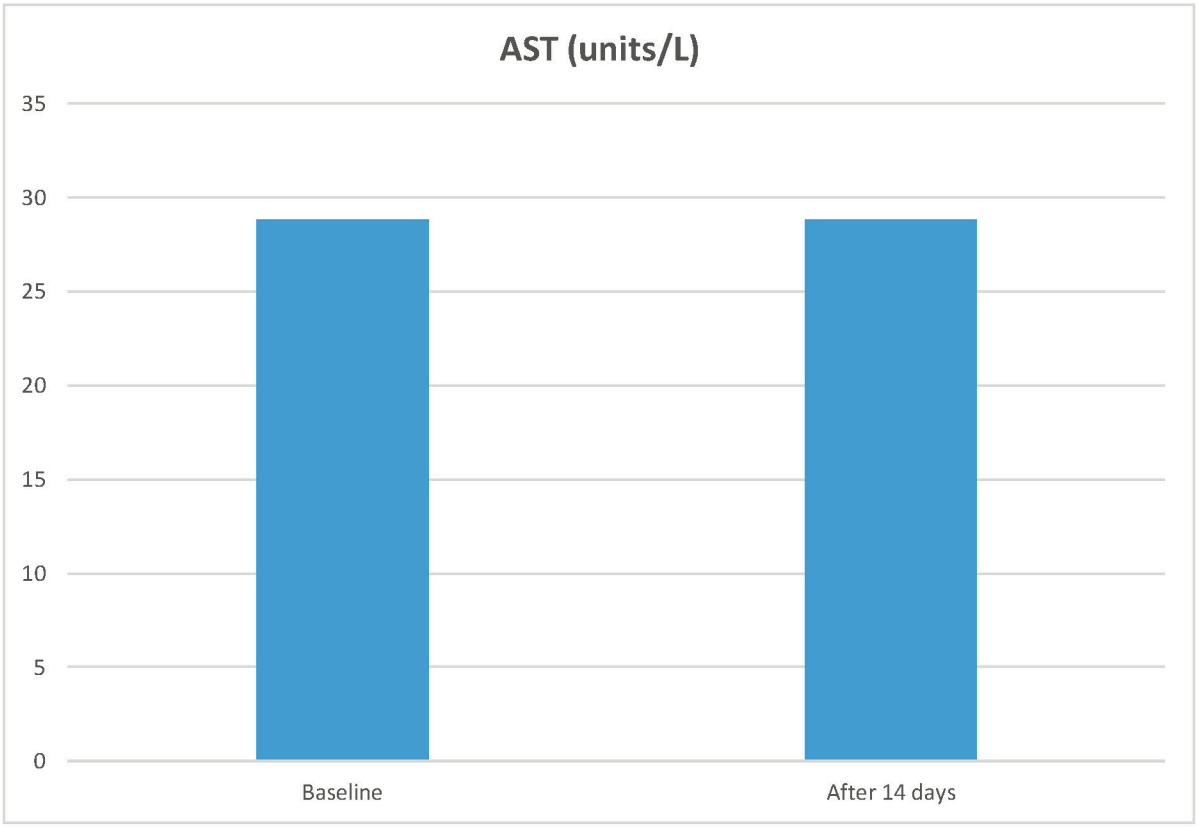


Figure 4.10: AST (units/L), Group A, Day 0 v/s Day 14, (N= 46)

Table 4.11: AST (units/L), Group B, Day 0 v/s Day 14, (N= 46)

	N	Mean	Std. Deviation	Std. Error Mean	P-Value
Baseline	46	29.59	4.440	.655	0.999^{NS}
After 14 days	46	29.59	4.440	.655	

Group B: treated with combination therapy (Lactobacillus reuteri + proton pump inhibitors), P value < 0.05: significant, *: Statistically significant, NS=Not Significant at 0.05. Test applied: Independent t-test.

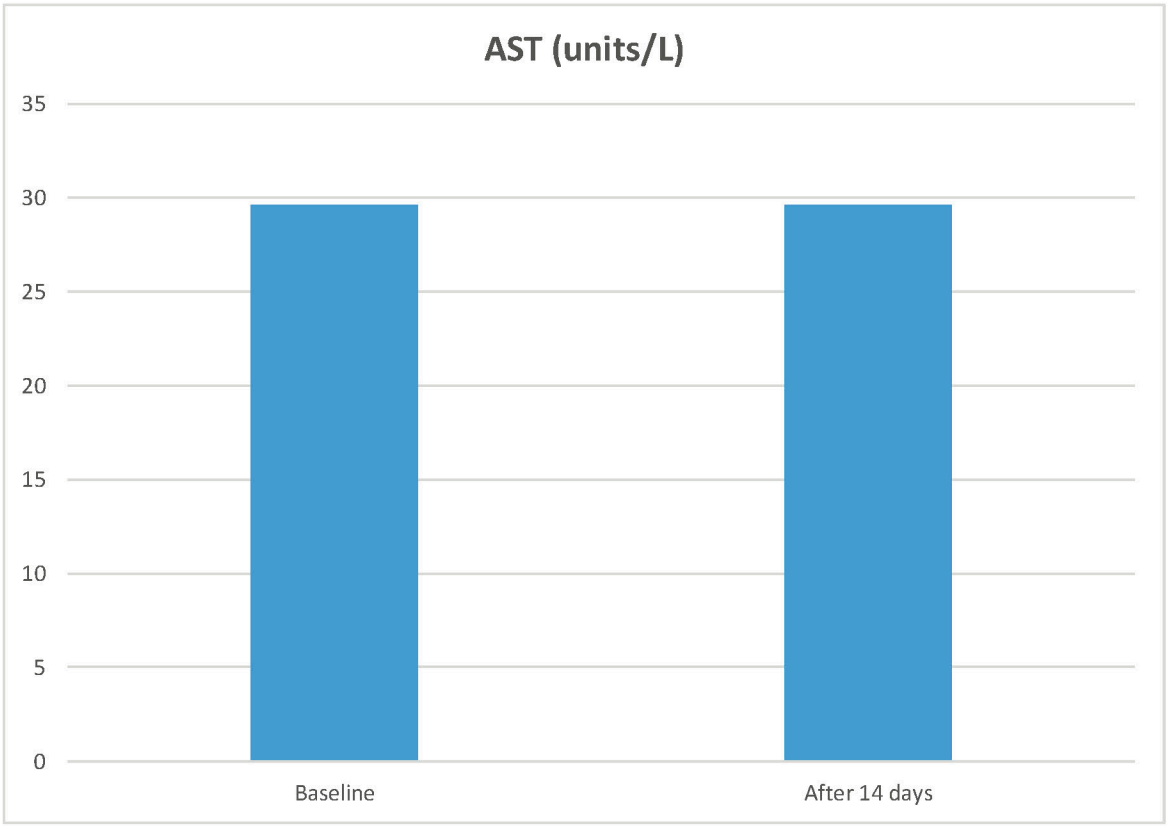


Figure 4.11: AST (units/L), Group B, Day 0 v/s Day 14, (N= 46)

Table 4.12: AST (units/L), between group A and group B (N= 92)

	N	Mean	Std. Deviation	Std. Error Mean	P-Value
GROUP A	46	28.83	5.305	.553	0.292^{NS}
GROUP B	46	29.59	4.416	.460	

Group A: treated with conventional triple therapy (Proton pump inhibitor + Clarithromycin + Amoxicillin); Group B: treated with combination therapy (Lactobacillus reuteri + proton pump inhibitors), P value < 0.05: significant, *: Statistically significant, NS=Not Significant at 0.05. Test applied: Independent t-test.

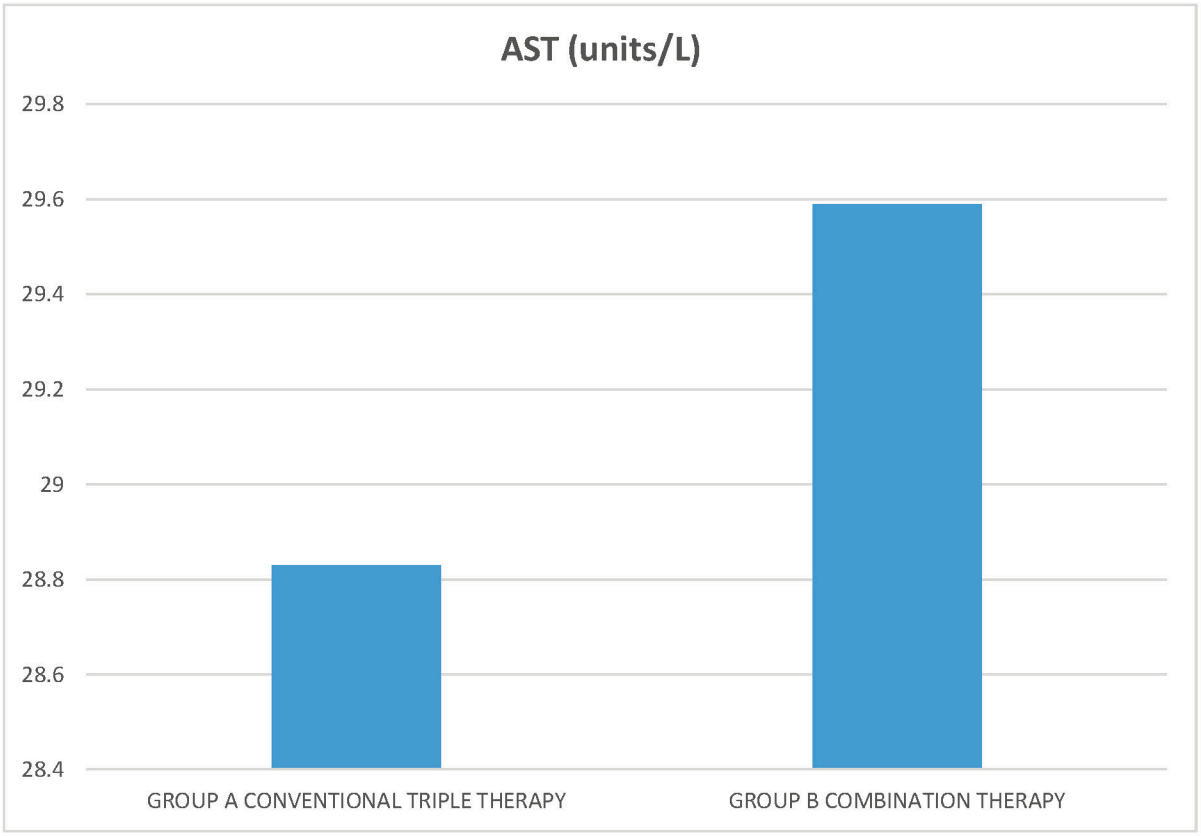


Table 4.12: AST (units/L), between group A and group B (N= 92)

4.3 Renal Function Test (Group A & B):

4.3.1 Serum Urea:

Mean Urea in group A at baseline and after 14 days was found to be same i.e. 15.43±2.208 mg/dL while mean Urea in group B at baseline and after 14 days was also same i.e. 15.24±1.923 mg/dL respectively. We found non-significant mean difference for Urea in group A at baseline and after 14 days (p=0.999). We found non-significant mean difference for Urea in group B at baseline and after 14 days (p=0.999). When we compared both the groups, group A and group B we also found non-significant mean difference at baseline and after 14 days (p=0.520) (Table 4.10, Table 4.11, Table 4.12, Figure 4.10, Figure 4.11 and Figure 4.12).

4.3.2 Serum Creatinine:

Mean Creatinine in group A at baseline and after 14 days was found to be same i.e. 0.83±0.167 mg/dL while mean Creatinine in group B at baseline and after 14 days was also same i.e. 0.83±0.163 mg/dL respectively. We found non-significant mean difference for serum creatinine in group A at baseline and after 14 days (p=0.999). We found non-significant mean difference for serum creatinine in group B at baseline and after 14 days (p=0.999). When we compared both the groups, group A and group B we also found non-significant mean difference at baseline and after 14 days (p=0.858) (Table 4.13, Table 4.14, Table 4.15, Figure 4.13, Figure 4.14 and Figure 4.15).

Table 4.10: Urea (mg/dL), Group A, Day 0 v/s Day 14, (N= 46)

	N	Mean	Std. Deviation	Std. Error Mean	P-Value
Baseline	46	15.43	2.208	.325	0.999^{NS}
After 14 days	46	15.43	2.208	.325	

Group A: treated with conventional triple therapy (Proton pump inhibitor + Clarithromycin + Amoxicillin), P value < 0.05: significant, *: Statistically significant, NS=Not Significant at 0.05. Test applied: Independent t-test.

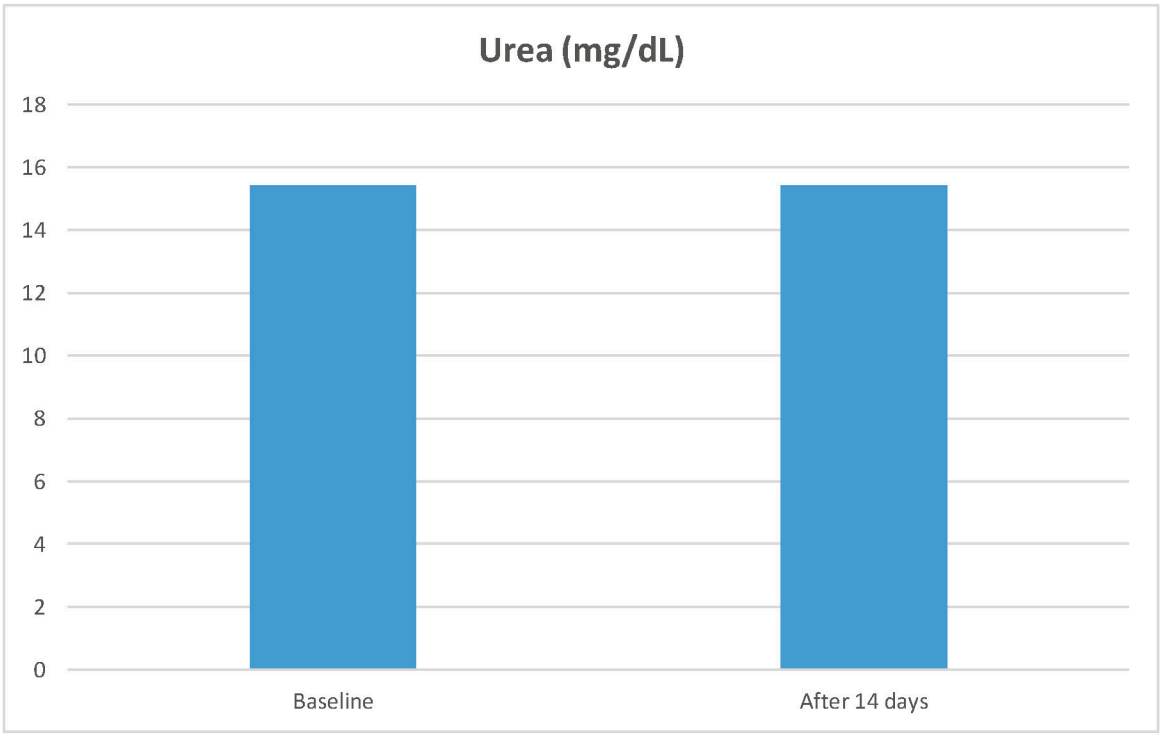


Figure 4.10: Urea (mg/dL), Group A, Day 0 v/s Day 14, (N= 46)

Table 4.11: Urea (mg/dL), Group B, Day 0 v/s Day 14, (N= 46)

	N	Mean	Std. Deviation	Std. Error Mean	P-Value
Baseline	46	15.24	1.923	.283	0.999^{NS}
After 14 days	46	15.24	1.923	.283	

Group B: treated with combination therapy (Lactobacillus reuteri + proton pump inhibitors), P value < 0.05: significant, *: Statistically significant, NS=Not Significant at 0.05. Test applied: Independent t-test.

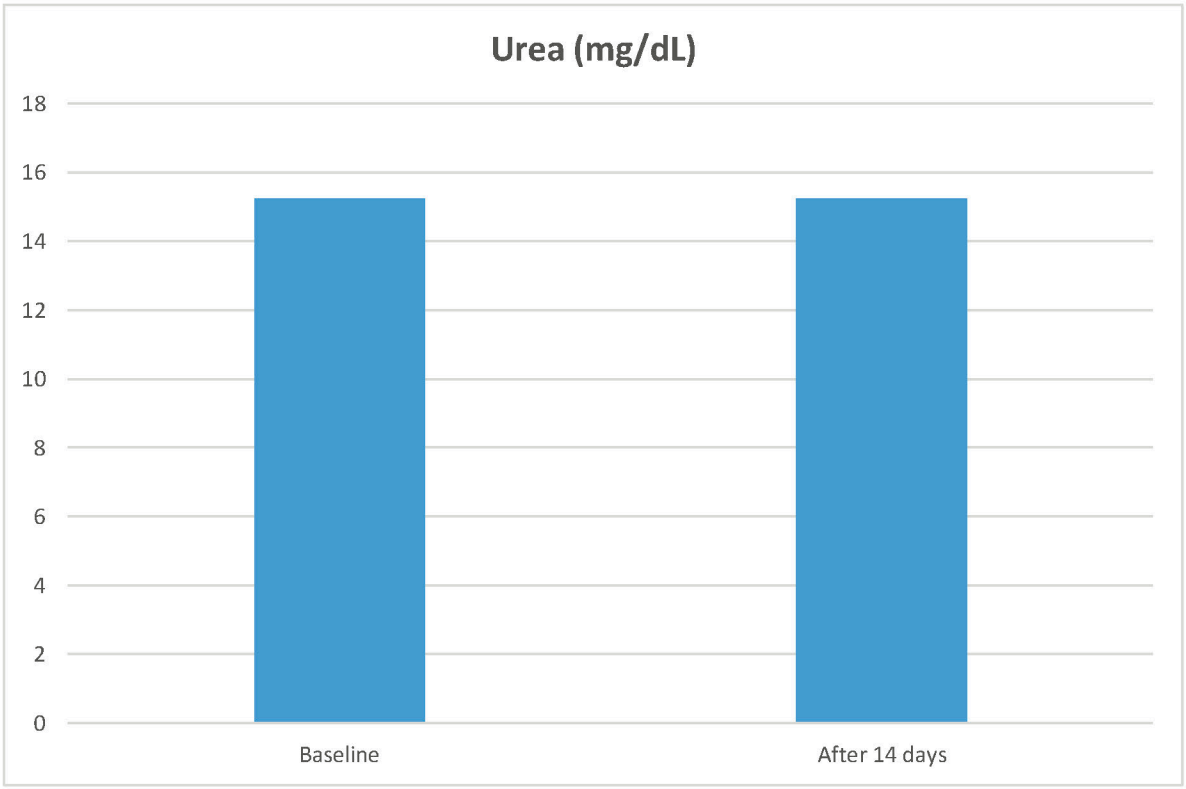


Figure 4.11: Urea (mg/dL), Group B, Day 0 v/s Day 14, (N= 46)

Table 4.12: Urea (mg/dL), between group A and group B (N= 92)

	N	Mean	Std. Deviation	Std. Error Mean	P-Value
GROUP A	46	15.43	2.195	.229	0.520^{NS}
GROUP B	46	15.24	1.912	.199	

Group A: treated with conventional triple therapy (Proton pump inhibitor + Clarithromycin + Amoxicillin); Group B: treated with combination therapy (Lactobacillus reuteri + proton pump inhibitors), P value < 0.05: significant, *: Statistically significant, NS=Not Significant at 0.05. Test applied: Independent t-test.

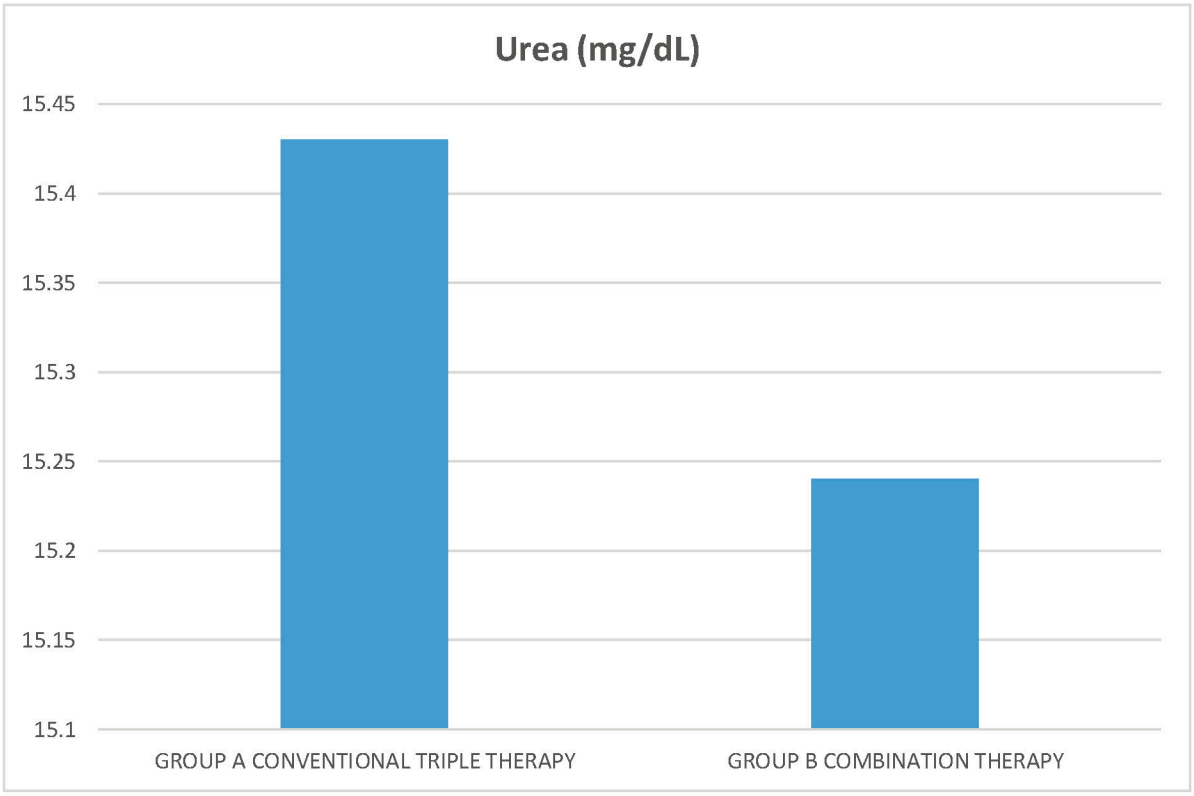


Figure 4.12: Urea (mg/dL), between group A and group B (N= 92)

Table 4.13: Creatinine (mg/dL), Group A, Day 0 v/s Day 14, (N= 46)

	N	Mean	Std. Deviation	Std. Error Mean	P-Value
Baseline	46	0.83	0.167	0.025	0.999^{NS}
After 14 days	46	0.83	0.167	0.025	

Group A: treated with conventional triple therapy (Proton pump inhibitor + Clarithromycin + Amoxicillin), P value < 0.05: significant, *: Statistically significant, NS=Not Significant at 0.05. Test applied: Independent t-test.

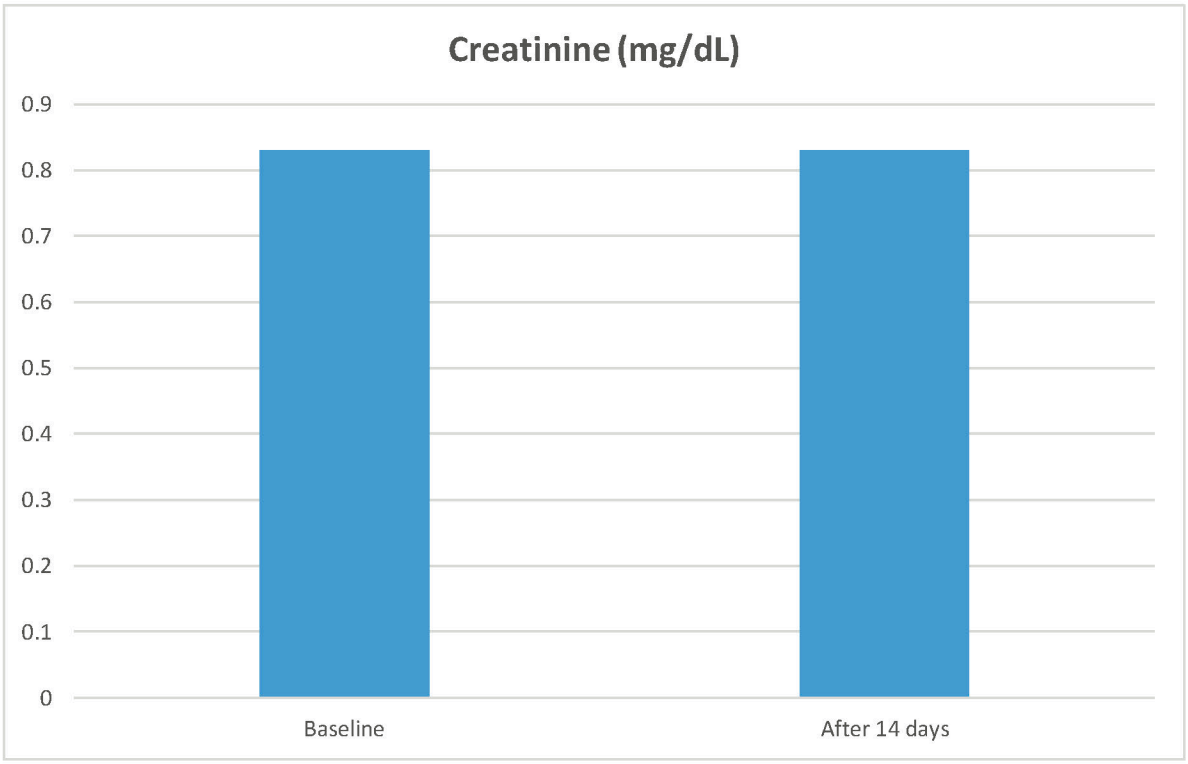


Figure 4.13: Creatinine (mg/dL), Group A, Day 0 v/s Day 14, (N= 46)

Table 4.14: Creatinine (mg/dL), Group B, Day 0 v/s Day 14, (N= 46)

	N	Mean	Std. Deviation	Std. Error Mean	P-Value
Baseline	46	0.83	0.163	0.024	0.999^{NS}
After 14 days	46	0.83	0.163	0.024	

Group B: treated with combination therapy (Lactobacillus reuteri + proton pump inhibitors), P value < 0.05: significant, *: Statistically significant, NS=Not Significant at 0.05. Test applied: Independent t-test.

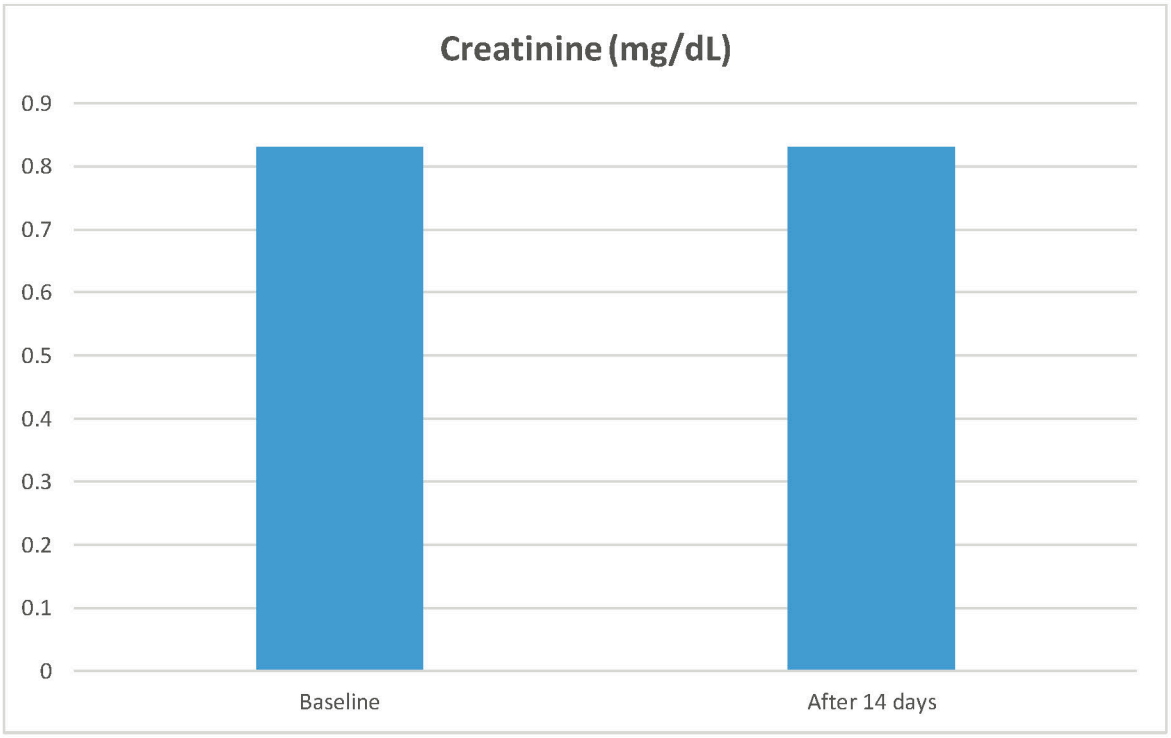


Figure 4.14: Creatinine (mg/dL), Group B, Day 0 v/s Day 14, (N= 46)

Table 4.15: Creatinine (mg/dL), between group A and group B (N= 92)

	N	Mean	Std. Deviation	Std. Error Mean	P-Value
GROUP A	46	0.83	0.166	.017	0.858^{NS}
GROUP B	46	0.83	0.162	.017	

Group A: treated with conventional triple therapy (Proton pump inhibitor + Clarithromycin + Amoxicillin); Group B: treated with combination therapy (Lactobacillus reuteri + proton pump inhibitors), P value < 0.05: significant, *: Statistically significant, NS=Not Significant at 0.05. Test applied: Independent t-test.

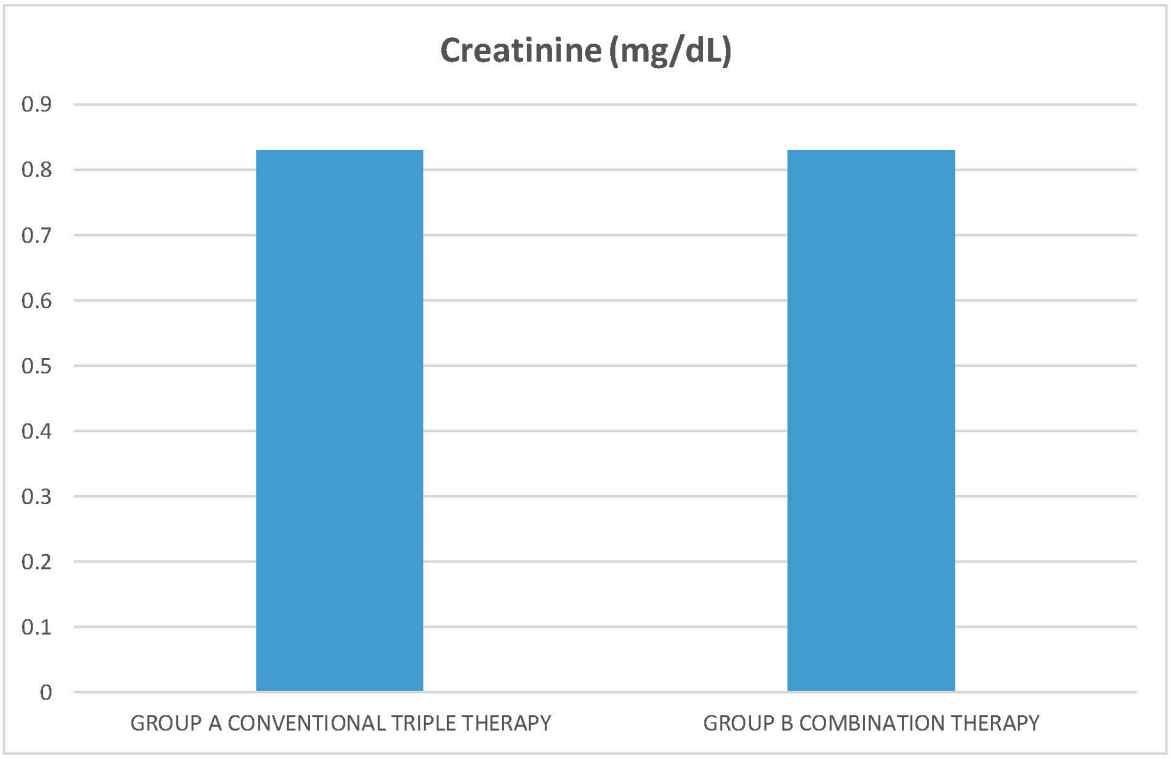


Figure 4.15: Creatinine (mg/dL), between group A and group B (N= 92)

4.4 Stool Antigen Test (Group A & B):

Stool antigen test was performed in group A which showed 56.5% positive test results and 43.5% negative test results. Stool antigen test was also performed in group B which showed 34.8% positive test results and 65.2% negative test results. When we compared both the groups, group A and group B we found statistically significant difference between the two groups (p=0.036) (Table 4.16 and Figure 4.16).

Table 4.16: Comparison between group A and group B (N= 92) (Day 14)

STOOL ANTIGEN * Group Cross tabulation

			Group		Total	P-value
			GROUP A CONVENTIONAL TRIPLE THERAPY	GROUP B COMBINATION THERAPY		
STOOL ANTIGEN TEST	Positive	Count	26	16	42	0.036*S
		% within Group	56.5%	34.8%	45.7%	
	Negative	Count	20	30	50	
		% within Group	43.5%	65.2%	54.3%	
Total		Count	46	46	92	
		% within Group	100.0%	100.0%	100.0%	

Group A: treated with conventional triple therapy (Proton pump inhibitor + Clarithromycin + Amoxicillin); Group B: treated with combination therapy (Lactobacillus reuteri + proton pump inhibitors), P value < 0.05: significant, *: Statistically significant, Test applied: Chi-square test.

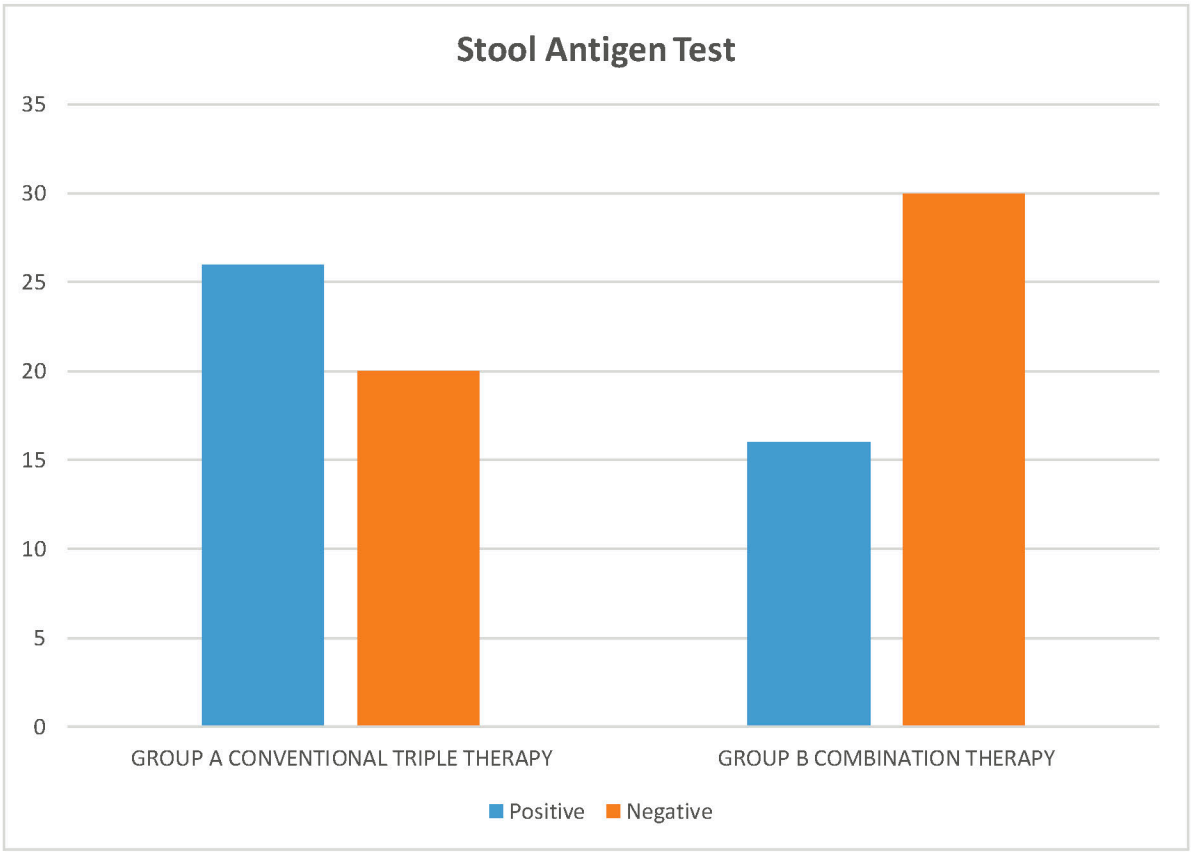


Figure 4.16: Comparison between group A and group B (N= 92)

4.5 Adverse Effects:

In group B none of the patients complained of any adverse effect. However, in group A 6 patients complained of heart burn (acidity) at the end of 14th day.

CHAPTER 5

DISCUSSION

The peptic ulcer disease can cause significant damage to the stomach mucosa and can lead to perforation and penetration of stomach mucosa and can even cause gastric carcinoma. Peptic ulcer is mainly caused by gram negative micro-organism *Helicobacter pylori* but there are others causes also like consumption of carbonated drinks, alcoholic beverages, spicy foods, smoking and regular use of NSAIDs. The most common complications are like stomach bleeding, penetration, perforation and fissure formation (Alhalabi *et al*., 2021). Therefore, to prevent these complications, eradication of *Helicobacter pylori* is very important. Although many treatment regimens have been proposed but conventional triple therapy is still considered as a main therapy in most part of the world. But unfortunately the effectiveness of this therapy is decreasing day by day due to resistance of antibiotics mainly clarithromycin and also due to the side effects caused by antibiotics (Murata *et al.*, 2020). So, because of these reasons there was a strong need to find out alternative treatment regimens. Many treatment regimens have been proposed but probiotics have found to be the most suitable ones according to previous researches held on different regions of the world. That is why the present study was aimed to identify the efficacy and safety of conventional triple therapy (Amoxicillin + Clarithromycin + Proton Pump Inhibitor) versus Probiotics Lactobacillus *reuteri* + Proton Pump Inhibitor combination therapy in Pakistani population having peptic ulcer disease.

Our primary objective was to assess the efficacy of conventional triple therapy i.e (Amoxicillin + Clarithromycin + Proton Pump Inhibitor) versus Combination therapy i.e. Probiotics (Lactobacillus *reuteri* + proton pump inhibitors) in peptic ulcer patients. Therefore, group A was recruited with 50 patients, who received Tab Amoxicillin of 1gm plus Tab Clarithromycin 500 mg and Tab Proton Pump Inhibitor

20 mg twice daily for 2 weeks and Group B was also recruited with 50 patients, who received Cap Lactobacillus reuteri 100 mg and Proton Pump Inhibitor 20 mg two times a day for 2 weeks after taking written informed consent. But after the initial visits four patients drop out of the treatment and did not come for the follow-ups due to unknown reasons. To find out which treatment regimen is more beneficial in treating the peptic ulcer disease we performed stool antigen test and then compared the results of both the groups (Group A and Group B) to find out how many patients have become *H. pylori* negative after completing the treatment. The results showed that the patients involved in group A after receiving the triple therapy out of 50 only 20 patients were tested negative (43.5%) and remaining 26 patients were tested positive (56.5%). While the patients in group B, after completing the treatment, out of 50, only 16 were remained positive (34.8%) and the rest of the 30 patients were tested negative (65.2%) and when we compared both the groups, the p-value was found to be as statistically significant (p-value = 0.036). It means that combination therapy is more effective than the conventional triple therapy. Hence, we can conclude from our finding that the combination therapy has more efficacy than the conventional triple therapy.

According to the findings of previous studies, many trials have shown that probiotics when compared to triple therapy or when used as an adjuvant to the triple therapy had shown better efficacy and the annihilation rate of *H. pylori* had also increased and it had also helped in minimizing the side effects. A prospective, randomized placebo trial was done between a period of November 2015 to November 2016 in Iran and they inspected the efficacy of probiotic in abolition of *H. pylori*. In this trial, 176 patients who were diagnosed with *H. pylori* disease after doing stool antigen test were enrolled. The study group was given the standard therapy along with addition of Lactobacillus probiotic for a duration of 4 weeks and the control group was given the placebo drug. The adverse effects of antibiotic therapy were recorded for every patient and six weeks later the termination of probiotic intake, all patients undergone the stool antigen test and follow-up was done after 6 months. The elimination rate of *H. pylori* infection was found to be significantly greater in probiotic group (78.4%), in comparison to placebo group (64.8%) (p=0.033). In addition, side effects were significantly less dominant in patients of probiotic group (p=0.047). So, this means that the probiotic group showed better efficacy and also

reduced the adverse effects (Haghdoost *et al*., 2017). Hence, this study is in harmony with our study.

Another retrospective study was conducted in South Korea from March 2013 to February 2014. In this study one group was given the STT along with a probiotic drug for a period of 1 week. The other CT group received STT plus metronidazole for 1 week. In this study both the groups displayed good results but the STT + probiotic group showed slightly better efficacy and also minimal side effects (Jung *et al.*, 2018). One more retrospective study was held in Japan between (January 2015 to December 2016) which showed similar results. In this study 468 patients were evaluated and they were classified into three groups, first PPI group (n= 150) was given (PPI, AMX and CLR), group VPZ (n= 271) was given (PPI, AMX and VPZ) and group PPI + MBM (n= 47) was given (PPI, AMX and CLR + probiotics). It was concluded that the abolition rate of *H. pylori* was significantly greater in PPI + MBM group (87.1%) as compared to PPI group (70.1%) (p = 0.0195). But, no significant difference with respect to adverse effects was found in all three groups (Mukai *et al*., 2020). Hence the study is consistent with our findings in terms of efficacy.

Rendering to a meta-analysis of five studies which included 484 pediatric patients. It was found that the probiotic (lactobacillus *reuteri*) group along with triple therapy showed more eradication rate of *H. pylori* as compared to the placebo group (1.19) [95% confidence interval (CI) 1.07–1.33]. The Lactobacillus group also showed decreased number of side effects especially diarrhea in children (RR = 0.30, 95% CI 0.10–0.85) (Fang *et al*., 2019). Another similar meta-analysis of twenty-nine trials involving 3122 participants containing 17 probiotic treatments were identified. When these trials were compared with control group it displayed that the *H. pylori* annihilation rate was higher in probiotic-associated triple therapy (RR) 1.19, 95% CI 1.13–1.25) and also minimizing the risk of adverse effects (RR 0.49, 95% CI 0.38–0.65) (Feng *et al*., 2017). Similar outcomes were detected by Hafeez *et al.* in his study. He observed that the combination of probiotics with the triple therapy enhanced the effectiveness up to 85% and the gastric symptoms were reduced from 76% to 15% (p=0.002) (Hafeez *et al*., 2021). Hence, the findings of these studies are in accord with our results. Similar results were detected in a randomized controlled trial in which 100 children who were between the age of 5 and 17 years were inducted

in the study. The children were classified randomly into two categories. First group was given standard triple therapy along with probiotic and second group was prescribed standard therapy alone. After experiment, they concluded that in group 1 the elimination rate was 88% in comparison to group 2 that is 72% (p = 0.046). They also suggested that the use of probiotic also reduced the adverse effects and improved the eradication rate (N Şirvan *et al.*, 2017).

Another meta-analysis of 40 studies had been done which included 8924 patients. In this analysis, the scientist compared the probiotic group with the control group and evaluated the effectiveness and safety of probiotic in abolition of *H. pylori*. The results showed that the probiotic group had greater rate of eradication in comparison to control group (RR 1.140, 95% confidence interval (CI) 1.101–1.180, P <0.001) and minimal side effects were analysed in probiotic group versus control group (RR 0.470, 95% CI 0.391–0.565, P<.001) (Shi et al., 2019). Hence the result of the meta-analysis is in consensus with our study. Similarly, another meta-analysis of 11 randomized controlled trial which comprised of 724 patients. In this trial, it showed that the probiotic (Lactobacillus) group had significantly greater elimination rate of *H. pylori* versus that of the control group (RR 1.16, 95% CI 1.08–1.25, P<0.0001). As for the adverse effects, Lactobacillus group had shown significantly reduced occurrence of side effects (RR = 0.36, 95% CI 0.17–0.74, P = 0.005) (Yu *et al.*, 2019). A further meta-analysis of 40 RCTs were conducted in which 5792 participants were recruited and previous articles were reviewed which was published between 2000 to 2019. In this review they evaluated the efficacy of probiotic when used as an adjuvant to standard therapy. After analysis they found that by adding the probiotic to the therapy the elimination rate of *H. pylori* has increased up to 10% as compared to the control group [odds ratio (OR), 1.94, 95% confidence interval (CI): 1.70–2.22, $P < 0.00001$]. It has also reduced the frequency of side effects and has improved the patient compliance (OR, 0.56, 95% CI: 0.45–0.70, $P < 0.00001$) (Zhang *et al.*, 2020). Hence, the findings are found to be in harmony with our study.

An additional meta-analysis of 100 and 40 studies was conducted by (Wang *et al*) involving 20,215 patients. In the study they compared the effectiveness of different probiotics in treatment of *H. pylori* disease. The result showed that the rate of elimination and the incidence of adverse effects were 84.1 and 14.4% in probiotic

group, while in the control group they were 70.5 and 30.1%. So, that means the probiotic group had better efficacy as compared to control group. The results of this analysis was similar to the previous studies (Wang *et al*., 2017). In the previous meta-analysis 30 RCTs were studied which aimed to analyse the efficacy of probiotic addition to the standard triple therapy in eradicating H. pylori. The results showed that the combination of probiotics significantly improved the abolition rates by 12.2% (relative risk [RR] =1.122; 95% confidence interval [CI], 1.091–1.153; P<0.001) and also it was found effective in both adults and children and all types of probiotics showed similar efficacy (Lau *et al*., 2016). So, this analysis also showed similar results with respect to our study.

Another comparative, prospective study was conducted in Bangalore which included 100 patients who were diagnosed with peptic ulcer disease. These participants were classified randomly into two categories. The patients in Group A were given the triple therapy i.e. amoxicillin 1 gm, lansoprazole 30 mg and clarithromycin 500 mg two times a day for 2 weeks and those present in group B were prescribed the probiotic 50 mg three times daily along with (pantoprazole 40 mg, amoxicillin 1 gm and clarithromycin 500 mg) twice a day for 2 weeks. After completion of treatment both groups were compared. The results showed that group B had more eradication rate in comparison to group A (84% vs 70%, p =0.096 at 95% CI) and the number of adverse effects were also found decreased in group B than that in group A (30% vs 46%, p=0.099). So, this means that the addition of probiotic was effective in the management of peptic ulcer (VD *et al*., 2016). Hence, the results are found to be in consensus with our study.

In a randomized placebo-controlled study 159 patients with positive *H. pylori* infection were enrolled in the trial. These participants were divided randomly into 3 groups and all patients were given the sequential therapy (amoxicillin 1gm + pantoprazole 40 mg twice a day) in first week, followed by (metronidazole 500 mg + clarithromycin 500 mg) in the second week but group 1 patients received additional probiotic supplement. Group 2 patients received placebo drug and group 3 patients received no supplement. The results showed higher eradication rate in group 1 than the rest of the groups (86.8% vs. 70.8%, p=0.025). Similarly, lower rate of side effects was observed in group 1 as compared to the other groups (1.88% vs. 12.26%,

p=0.036) (Cekin *et al*., 2016). This study also showed similar findings as compared to our study.

Next study of 13 randomized controlled trials comprising of 2306 patients also showed the similar results. In that trials the probiotic supplementation group showed significantly greater annihilation rate as compared to control group [RR 1.15, 95% confidence interval (CI): 1.10±1.20, P<0.00001] and also the probiotic group showed minimal adverse effects versus that of control group (RR = 0.71, 95% CI: 0.54±0.94, P = 0.02) (Lü et al., 2016). Similarly, previous studies also observed the efficacy and safety of probiotic as an adjuvant therapy. That meta-analysis included 21 RCTs comprising of 3,814 participants. The analysis showed that the probiotic group has better efficacy as compared to placebo group (RR, 1.12; 95% confidence interval (CI), 1.06-1.19] and the adverse effects was also found to be decreased in probiotic group (RR, 0.60; 95% CI, 0.40-0.91) (Lv *et al*., 2015). In an earlier review of 45 RCTs which included 6997 participants, similar results were observed. It was noted that when probiotic was combined with triple regimen the elimination rate was significantly increased (RR = 1.13; 95%CI: 1.10-1.16; P < 0.001). It was also observed that the incidence of adverse effects was also reduced when the combination therapy was given (RR = 0.59; 95%CI: 0.48-0.71; P < 0.001) (Zhang *et al*., 2015). Hence, the outcomes of these analysis are in consensus with our study.

Likewise, a scientist Hauser *et al*. 2015 conducted a multicentre, prospective, double-blind, placebo-controlled trial to find out the effectiveness of probiotics as an additional remedy in the management of *H. pylori* disease. In this study 650 individuals were added and they were prescribed standard triple therapy along with probiotic or placebo for a duration of 2 weeks. The probiotic groups showed better eradication rate (87.38%) as compared to placebo group (72.55%) and it also showed minimal side effects in relation to placebo group (Hauser et al., 2015). In an another meta-analysis of 23 studies which consisted of 3900 participants. The results showed that the patients in control group who received triple therapy alone had lower eradication rate (72.26%; 95% CI, 67.66%-74.13) than that of treatment group who received triple therapy plus probiotic (80.74%; 95% CI, 74.68%-82.76%). Patients in treatment group had shown lower incidence of adverse effects too (Gong *et al*., 2015). Hence, the findings are in harmony with our study.

In the previous meta-analysis conducted by Li *et al.* 2014 similar results were seen. That meta-analysis comprised of 7 studies which included 508 pediatric participants. The result of this trial was found that the eradication rate of probiotic group was (1.96) (95 % CI 1.28–3.02) versus the control group (2.25) (95 % CI 1.41–3.57) and when the side effects were compared between both the groups the result was 0.32 (95 % CI 0.13–0.79) (Li *et al*., 2014). In another meta-analysis also revealed similar results. In that review the annihilation rate of H. pylori in probiotic group was found to be 1.67 (95% CI: 1.38-2.02) versus the control group 1.68 (95% CI: 1.35-2.08). The side effects were also observed to be less in probiotic group than that of control group (OR = 0.49, 95%CI: 0.26-0.94) with significant heterogeneity (I^2= 85.7%) (Zhu *et al*., 2014). Hence the findings of both the reviews are in accordance with our study. In a double-blind, placebo-controlled, randomized clinical trial which was conducted in Egypt between June 2012 to February 2013. In this trial, 70 participants were recruited and they were randomly assigned into two groups. The patients in group A were given conventional therapy for 2 weeks along with *L. reuteri* while those in group B were prescribed placebo drug for 4 weeks. The *L. reuteri* group had more eradication rate (74.3%) than the placebo group (65.7%). Also, the first group showed less number of side effects versus the group that was treated with placebo (Emara *et al*., 2014). So this study also supports our findings.

A prospective, randomized open label clinical test was carried out to investigate the outcome of probiotic in eliminating the *H. pylori* infection by adding in various therapies. The participants were classified into four categories. Group A included 106 patients it was a control group who were treated with conventional triple regimen, Group B included 100 patients who were given STT along with probiotic, Group C included 95 patients they were given probiotic 2 weeks before the STT and Group D had 76 patients who received probiotic along with sequential therapy. The results showed that the annihilation rate in group A was 68.9%, in group B was 83% ($P < 0.001$), in group C was 90.5% and in group D was 90.8%. So, from this study it was concluded that the addition of probiotic improved the cure rate (Dajani *et al*., 2013).

In a previous open randomized controlled trial which consists of 234 patients and this study was held between November 2008 to July 2009. The participants were

classified randomly into three categories. One class which was OCA group received the standard therapy for a period of 7 days. Second class which was POCA group in this the patients were prescribed first the probiotics then triple therapy for 1 week. Third class was OCAP group which was given first the standard therapy for the same duration and then the probiotic for a period of 2 weeks. The results showed that the elimination rate was much greater in the POCA (62/76; 81.6%, 95% CI 72.8%-90.4%) and OCAP group (61/74; 82.4%, 95% CI 73.6%-91.2%) in comparison to the OCA group (48/79; 60.8%, 95% CI 49.9%-71.7%), (P = 0.014 and P = 0.015) and these two groups also helped in minimizing the adverse effects (Du *et al*., 2012). Therefore, the results of these studies are in harmony with our findings.

In a prospective observational study which is held at Columbia Asia Hospital Medan from August 2019 – April 2020 the effectiveness of probiotic along with triple therapy was observed in children who were diagnosed with H. pylori gastritis. For diagnosis stool antigen test and endoscopy was done. The children were classified randomly into treatment and control group. The children who are included in treatment group were given triple therapy plus probiotic and those present in control group were given triple therapy alone twice a day and after 4 week's follow-up was done. The results showed that out of 33 children, 31 patients were cured in treatment group and out of 33 children, 29 patients were cured in control group and when side effects were observed out of 33 children only 6 developed side effects in treatment group and 22 children developed side effect in control group. So, it was concluded that there was no significant change between treatment and control group (p = 1,000). Though, there was significant difference in minimizing the side effects in treatment group (p = 0.015) (Nasution *et al*., 2020). Hence, the study showed inconsistent results according to our study with respect of efficacy.

A systemic review with pooled-data analysis of 11 studies was conducted by (Losurdo *et al*., 2018). In this review they analysed the elimination of H. pylori by giving the probiotic monotherapy. This showed that out of 403 cases probiotic eliminated H. pylori in only 50 patients. The mean eradication rate was found to be 14% (95% CI: 2%-25%, p =0.02). When probiotic was compared with placebo it showed results in favour of probiotics (95% CI: 2.97-21.05, p < 0.001). But when adverse effects were compared no significant difference was found (OR = 1, 95%CI:

0.06-18.08) (Losurdo *et al.*, 2018). Hence, the findings are inconsistent with respect to our study.

In a meta-analysis of 21 RCTs were done which included 3,520 participants. In this review the efficacy of probiotic plus standard therapy versus placebo was observed and it was concluded that the probiotic group did not improve the eradication rate as compared to placebo group (OR 1.44, 95% CI: 0.87, 2.39) (Lu *et al.*, 2016). Hence, this review showed contradictory results to our study.

In a prospective, double-blind, placebo-controlled trial, 107 individuals were classified randomly into two categories. One group of 52 patients were prescribed therapy (30 mg of lansoprazole, 500 mg of tetracycline and 200 mg of furazolidone) for 7 days twice daily and other group of 55 patients were prescribed probiotic compound twice a day for 30 days. Follow-up was done after the treatment was completed. The findings showed that the elimination rate of probiotic group was 89.8% and of placebo group was 85.1% ($p = 0.49$). After 7 days the rate of adverse effects of probiotic group was found to be 59.3% and that of placebo group was 71.2% ($p = 0.20$). When the adverse effects were assessed after 30 days it was 44.9% in treatment group and 60.4% in placebo group ($p = 0.08$). Hence, it was concluded that there was no dissimilarity between probiotic and placebo group in terms of efficacy and adverse effects (Navarro-Rodriguez *et al.*, 2013). In another prospective, double-blind placebo controlled study similar results were found. The results of that study showed that probiotic (*L. reuteri*) combination alone did not increase the elimination rate of *H. pylori* (Francavilla *et al.*, 2014). Therefore, the findings are inconsistent with respect to our study.

A single-blind, interventional and treatment-efficacy study was done in Portugal which included 62 peptic ulcer patients and were classified randomly into two categories. Group 1 was prescribed (esomeprazole 20 mg, amoxicillin 1000 mg and clarithromycin 500 mg) two times regularly for a period of 8 days (EAC) and the second group was given probiotic (L. *acidophilus*) two times regularly for 8 days (EACL). Follow-up was done after 6 weeks. The results showed that there was no significant change between the two groups in terms of eradication rate (EAC = 80.6%; EACL = 83.9%, $p=0.740$). So it was concluded that adding probiotic to triple regimen

did not increase the elimination rate (Silva Medeiros *et al.,* 2011). Hence, the outcomes of the study are not consistent with our findings.

Our secondary objective was to measure the safety of conventional triple therapy i.e (Amoxicillin + Clarithromycin + Proton Pump Inhibitor) versus Combination therapy i.e. Probiotics (Lactobacillus *reuteri* + proton pump inhibitors) in peptic ulcer patients. To find out the safety of drugs involved in conventional triple therapy and combination therapy we checked different parameters like complete blood count, liver function test and renal function test.

Our first parameter was to find out whether the conventional triple therapy and combination therapy changes the hemoglobin level in the patient's body. So, to check this we performed the complete blood count test in both the groups (Group A and Group B). The test showed insignificant mean difference in both the groups at baseline and after 14 days and the p-value was also found to be insignificant between both the groups (0.741). In the previous study, which was experimented on 100 individuals who were detected with H. pylori gastritis after doing stool antigen test. In this study, participants were classified into two categories: Group A comprised of 50 patients who were prescribed amoxicillin, clarithromycin and esomeprazole for 2 weeks and Group B which also included 50 patients and also received similar drugs as Group A but there was an addition of vitamin C tablet as supplement for 4 weeks. This experiment also showed no significant change in both the groups after treatment and the mean Hb value in group A was 13.20 ± 0.92 and in group B was 12.77 ± 1.19. It also showed insignificant p-value between both the groups (p-value 0.402) (Shahawy *et al*., 2020).

A former study conducted by Hassan *et al*., 2019 also showed no significant change between the two groups before and after treatment. The mean hemoglobin value was found to be 12.5 ± 1.88 in group 1 and 12.82 ± 1.58 in group 2 and it also showed non-significant p-value (0.37) (Hassan *et al.,* 2019). Another pilot double-blind randomized trial was conducted in South-East of Nigeria which enrolled 63 patients from January 2017 until August 2017 also showed similar results. In group 1 which received Syferol-IHP with CTT the mean Hb concentration was found to be

11.9±2.1 at baseline and 12.2±3.4 after treatment. In group 2 which received placebo with CTT the mean Hb value was 12.9±2.8 at baseline and 12.3±3.1 after treatment and both group showed insignificant p-value (0.909) (Eleje *et al*., 2019). But a study conducted by Mokhtare *et al.* showed contradictory results. This study showed significant difference in the Hb level after treatment by standard triple therapy (5.37 _ 0.52 vs 5.25 _ 0.53, p = 0.006) (Mokhtare *et al.*, 2021).

Next, we had identified the safety profile of these treatment regimens by liver function test (LFT), as, drug induced-liver injury has been found in many previously reported studies. The identification of safety of conventional triple therapy and combination therapy in hepatocellular hepatotoxicity was manifested by serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST). We have found clinically insignificant results in both the treatment groups. The mean value of ALT was normal in all patients of both groups and it showed no significant difference between both the groups (p-value = 0.714). Next, the mean values of AST in group A and B was also found to be normal and within normal range. It also showed no significant change between the two groups (p-value = 0.292). An earlier study conducted by Hassan *et al.*,2019 also showed no significant difference between the two groups before and after treatment. The mean ALT value was found to be 20.45± 9.67 in group 1 and 17.9± 5.44 in group 2 and it also showed non-significant p-value (0.32). Similarly, the mean AST value in group 1 was found to be 21.56±7.59 and 21.32±7.77 in group 2 with insignificant p-value (0.75) (Liou *et al*., 2019). Another past study also showed similar findings to our results. This trial also showed no significant change in both Group A and Group B after treatment and the mean ALT and AST value in group A was 23.0 ± 7.80 and 25.0 ± 7.10 and in group B was 22 ± 5.70 and 27 ± 6.87. It also showed insignificant p-value of ALT in both the groups (0.432) and the p-value of AST was also not statistically significant (0.610) (Shahawy *et al*., 2020).

An open-angle, parallel group, randomized-controlled pilot study was conducted in India from June 2017 to December 2018. In this study the 333 patients who were tested positive with H. pylori infection were enrolled. The patients were classified randomly into two categories. One group was given standard management

therapy (SMT) and other group was given H. pylori-eradication therapy (HPET). The analysis of data was done at baseline and after 24 weeks. At baseline the value of ALT was found to be 45 IU/L and after 24 weeks the value was 36 IU/L in SMT group and in HPET the value of ALT at baseline was 53 IU/L and after 24 weeks the value was 36 IU/L. The p-value was found to be insignificant between the two groups (0.583). At baseline the value of AST was found to be 32 IU/L and after 24 weeks the value was 28 IU/L in SMT group and in HPET the value of ALT at baseline was 53 IU/L and after 24 weeks the value was 42 IU/L. The p-value was found to be insignificant between the two groups (0.775). It showed that there was no change in the liver function parameter between the two groups (Maharshi *et al*., 2020). Hence, the finding was consistent with our study.

Next, we had identified the safety profile of these treatment regimens by renal function test (RFT). Regarding we had checked the serum urea and serum creatinine level and we found out that there was no change in the serum urea and serum creatinine level in both group A and group B at baseline and after 14 days of treatment and it showed insignificant p value for urea (p=0.520) and also for creatinine (p=0.858). This means that both the groups show no significant change in the serum urea and serum creatinine level after taking the conventional triple therapy and combination therapy. Our findings are in accord with the previous study performed by Hassan *et al*., 2019 which also showed no significant change between the two groups before and after treatment. The mean serum urea value was found to be 21.4 ± 7.10 in group 1 and 22.86 ± 7.75 in group 2 and it also showed non-significant p-value (0.36). Similarly, the mean serum creatinine value in group 1 was found to be 0.77 ± 0.24 and 0.78 ± 0.23 in group 2 with insignificant p-value (0.81). Another past study also showed similar findings to our results. In this study, group 1 which received Syferol-IHP with CTT the mean serum urea value was found to be 4.5±2.1 at baseline and 4.8±3.6 after treatment. In group 2 which received placebo with CTT the mean serum urea value was 5.4±8.7 at baseline and 3.9±1.4 after treatment and both group showed insignificant p-value (0.258). Similarly, the mean serum creatinine value in group 1 was found to be 102.5±25.1 at baseline and 93.2±29.8 after treatment and in group 2 the value was 94.3±15.5 at baseline and 92.3±26.9 after treatment with insignificant p-value (0.907) (Khatun *et al*., 2020).

CHAPTER 6

CONCLUSION

6.1 Conclusion of the study:

It is concluded that:

a) Probiotic (Lactobacillus *reuteri*) is clinically effective for eradication of *Helicobacter pylori* to treat peptic ulcer disease.

b) Probiotic (Lactobacillus *reuteri*) has also proven to be effective in preventing the side effects associated with antibiotics in conventional therapy.

c) Combination therapy (Group B) has shown better efficacy and safety profile as compared to conventional triple therapy (Group A).

6.2 Recommendations:

It is recommended that

i. Probiotics should be used as an adjuvant therapy along with triple regimen for complete eradication of *H. pylori* bacteria.

ii. Future studies are necessary to find out the benefits of probiotics in other gastrointestinal problems.

iii. Probiotics can safely be prescribed in hepatic and renal patients as it has shown beneficial effects.

iv. Future studies should be conducted to observe its cost-effectiveness on patients of peptic ulcer with other commonly prescribed commercially available drugs.

6.3 Strengths of the study:

a. Combination therapy have shown better efficacy and also helped to restore the normal microflora in the stomach.

b. This therapy has shown minimal side effects which improved the patient compliance.

c. Safety profile with respect to hepatic, renal, and hemoglobin were closely observed before start and at the end of treatment and have found to be good.

d. To the best of our knowledge no study was conducted on Lactobacillus *reuteri* in Pakistan according to the literature search of past ten years.

6.4 Limitations of the study:

i. Single centric study

ii. Number of patients were less.

iii. The designed time period to study the effect was short.

iv. Results of the study cannot be generalized.

v. Endoscopy could not be done due to budget restraints.

CHAPTER 7

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A) FRC APPROVAL LETTER

Bahria University
Discovering Knowledge
Medical and Dental College, Karachi

Ref no: FRC/BUMDC -13/2020-Pharm-118 **MS-11**

Approval of Research Proposal

Mr/Miss/Ms/Mrs/ **Dr. Hina Amjad**

Registration No:

Dear MS/MPhil Student,

I am pleased to inform you that your research proposal on **"Evaluation Of Triple Therapy Versus Probiotic (Lactobacillus Reuteri) - Proton Pump Inhibitor Combination In Eradication of Helicobacter Pylori"** has been approved. You may, therefore, continue your research on this theme and produce a quality thesis, as per the HEC requirements.

I take this opportunity to remind you that you must complete your thesis, and defend it successfully, by SPRING 2021; this is the date which marks the end of the Extended Duration of your programme. However, to remain eligible for honours and awards, you must complete the thesis, and successfully defend it, by the end of 10 week of final semester.

I wish you every success.

Dated: 30/09/20

CHAIRPERSON FRC, BUMDC

Distribution:

- DG
- Principal
- Student's File (with the HOD/PGP Coordinator)
- Student

B) ERC APPROVAL LETTER

BAHRIA UNIVERSITY MEDICAL AND DENTAL COLLEGE

Defence phase II, Sailor Street, adjacent to PNS Shifa, Karachi. Tel: 021-35319491-9

ETHICAL REVIEW COMMITTEE

LETTER OF APPROVAL

Date: 15-Jan-21

FRC Reference:
FRC/BUMDC -13/2020-Pharm-118

PATRON
Prof. Ambreen Usmani
Principal & Dean
Health Sciences(BU)

CHAIRPERSON
Dr. Quratulain Javaid

SECRETARY
Dr. Ambreen Surti

MEMBERS
Prof M Alamgir
Prof Anis Jafarey
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Prof Reza H Syed
Ms Shabina Arif
Mr M Amir Sultan
Surg Lt Cdr Farah
Surg Lt Cdr Sadia

Dr. Hina Amjad

Subject: Institutional Approval of research study

Title of Study: "Evaluation of Triple Therapy Versus Probiotic (Lactobacillus Reuteri) - Proton Pump Inhibitor Combination in Eradication of Helicobacter Pylori "

Principal Investigator: Dr. Hina Amjad

Reference No: ERC 36/2021

Dear Dr. Hina Amjad,

Thank you for submitting the above mentioned study proposal. ERC Bahria University Medical and Dental College has reviewed this project in the meeting held on 14-Jan-2021 and gives approval. Kindly notify us when the research is complete.

Regards,

15/1/21

DR. AMBREEN SURTI
Secretary, ERC
BUMDC

15/1/2021

DR. QURATULAIN JAVAID
Chairperson, ERC
BUMDC

Cc:
DG-BUMDC
Principal BUMDC
Chairperson ERC

C) SUBJECT CONSENT FORM (ENGLISH VERSION)

You are giving your consent to participate voluntarily and at your own will in this research clinical trial project that aims to treat your disease of peptic ulcer by giving drugs:

(A) Triple therapy (Proton pump inhibitor 20 mg BD + Clarithromycin 500 mg BD+ Amoxicillin 1 gm BD) per orally for 14 days.

(B) Cap Lactobacillus reuteri 100 mg BD + proton pump inhibitor 20 mg BD per orally for 14 days.

You have been told the possible side effects of the drugs (A). These can cause headache, diarrhea, constipation nausea, vomiting and abdominal discomfort.

You have been told the possible side effects of the drugs (B). These can cause diarrhea, vomiting, indigestion, abdominal pain and loss of appetite.

You have been explained in detail the nature and significance of participating in the project and you understand the provided explanation.

You have been told that findings of your disease and your data will be kept strictly confidential and will be used only for the benefit of community, publications and paper presentations.

You have been explained that laboratory investigations will be conducted for the diagnosis and start of drug therapy and to monitor the therapy and side effects of the given drugs. For this purpose, you fully agree to give your blood samples at the beginning and end of study and when required in between.

You also agree to give all relevant information needed, in full and to the best of your knowledge to the researcher. It is clarified to you that no incentive will be provided to you for participating in the study except the cost of X-ray lab investigations and drugs, whereas you do have the right to withdraw from the study at any time.

You are advised to contact Dr. Hina Amjad on mobile number: 0323-2110919 or visit National Medical Centre in case of any query/ emergency related to your disease.

Name of Patient: _____ S/D/W/0_____
Treatment Assigned to Patient: _____
Signature / Thumb impression of Patient: _____
Name of Researcher: _____
Signature of Researcher: _____
Date: _____

URDU VERSION

(مریض کے لئے اجازت نامہ)

آپ رضا کارانہ طور پر اپنی مرضی سے اس کلینیکل ٹرائل پروجیکٹ میں حصہ لے رہے ہیں جس کا مقصد ادویات کے ذریعے آپ کے Peptic ulcer (میدے کا السر) کی بیماری کا علاج کرنا ہے۔

(A) ٹرپل تھراپی ٹیبلٹ پروٹون پمپ انہیبیٹر 20 ملی گرام بی ڈی + ٹیبلٹ کلریثرومائسن 500 ملی گرام بی ڈی + ٹیبلٹ اموکسیلین 1 گرام بی ڈی 14 دنوں کے لئے استعمال کرنی ہے۔

(B) کیپسول لیکوباسلس ریٹری 100 ملی گرام + ٹیبلٹ پروٹون پمپ انہیبیٹر 20 ملی گرام بی ڈی 14 دنوں کے لئے استعمال کرنی ہے۔

آپ کو ادویات (A) کے ممکنہ مضر اثرات کے بارے میں بتایا گیا ہے۔ یہ سر درد، اسہال، قبض، متلی، الٹی اور پیٹ میں تکلیف کا سبب بن سکتی ہے۔

آپ کو ادویات (B) کے ممکنہ مضر اثرات کے بارے میں بتایا گیا ہے۔ یہ اسہال، الٹی، بدضمی، پیٹ میں درد اور بھوک میں کمی کا سبب بن سکتی ہے۔

آپ کو تحقیقاتی عمل میں حصہ لینے کی نوعیت اور اہمیت کے بارے میں تفصیل سے آگاہ کیا گیا ہے اور آپ فراہم کردہ معلومات اور وضاحت کو سمجھتے ہیں۔

آپ کو بتایا گیا ہے کہ آپ کی بیماری اور آپ کے اعداد شمار کو سختی سے خفیہ رکھا جائے گا اور صرف کمیونٹی کے فائدے اور آرٹیکل کے اشاعت کے لئے استعمال کیا جائے گا۔

آپ کو بتایا گیا ہے کہ بیان کردہ لیبارٹری تحقیقات، تشخیص اور ادویات کی تھراپی کا آغاز اور دی گئی ادویات کے ضمنی اثرات کی نگرانی کے لئے کی جائیں گی۔ اس مقصد کیلئے آپ اپنے خون کے نمونے۔ تحقیق کے آغاز، درمیان اور اختتام پر، جب بھی ضرورت ہو، پوری طرح دینے پر اتفاق کرتے ہیں۔

آپ پوری طرح سے اور بہتر علم کے ساتھ محقق کو تمام متعلقہ معلومات دینے پر اتفاق کرتے ہیں۔ یہ آپ کو واضح کیا گیا ہے کہ ایکس رے، لیبارٹری کی تحقیقات اور ادویات کی قیمت کے علاوہ تحقیق میں حصہ لینے کے لئے آپ کو کوئی ادائیگی نہیں کی جائے گی، جبکہ آپ کو کسی بھی وقت تحقیقاتی عمل کو چھوڑنے کا حق حاصل ہے۔

آپ کو مشورہ دیا جاتا ہے کہ ڈاکٹر حنا امجد کو موبائل نمبر 0323-2110919 پر رابطہ کریں، آپ کی بیماری سے متعلق کسی بھی سوال/ہنگامی صورت حال میں نیشنل میڈیکل سینٹر (NMC) سے رابطہ کریں۔

مریض کا نام:_____ **والد/شوہر کا نام:**_____

مریض کا علاج:_____ **مریض کے دستخط/انگوٹھے کا نشان:**_____

ڈاکٹر کا نام:_____ **ڈاکٹر کے دستخط:**_____

D) SUBJECT EVALUATION FORM

Serial No: _____ Reg. No: _____ Date: _____

Patient's Name: _____S/D/W/O _____

Age: _____ Sex: _____

Address: _____

Phone No.: _____ Occupation: _____

Presenting Complaints:

Epigastric Pain	(YES)	(NO)
Nausea / Vomiting	(YES)	(NO)
Abdominal fullness	(YES)	(NO)
Loss of appetite	(YES)	(NO)

Past Medical History:

Other Co-morbidity

Family History: _____

PHYSICAL EXAMINATION AT THE TIME OF REGISTRATION

Blood Pressure **Temperature** **Pulse**

Abdomen: _____

Chest:

BASELINE LABORATORY FINDINGS

CBC: Hb: _____ ALT: _____ AST: _____

Urea: _____ Creatinine: _____

BASELINE ASSESSMENT OF SYMPTOMS

(Day-0)

Date: _____

Parameters Evaluation:

1. Epigastric pain (YES) (NO)
2. Improvement in appetite (YES) (NO)
3. Stool Antigen Assay (POSITIVE) (NEGATIVE)

PATIENT'S FOLLOW-UP RECORD

Follow-up Visit (14 DAYS)

Date: _____

Parameters Evaluation:

1. Epigastric pain (YES) (NO)
2. Improvement in appetite (YES) (NO)
3. Stool Antigen Assay (POSITIVE) (NEGATIVE)

Adverse Effects:

1. Nausea (YES) (NO)
2. Vomiting (YES) (NO)

3. Headache (YES) (NO)

4. Rash (YES) (NO)

5. Diarrhea (YES) (NO)

6. Stomach pain (YES) (NO)

7. Heart burn (acidity) (YES) (NO)

8. Taste disturbance (YES) (NO)

LABORATORY FINDINGS

Follow-up Visit (14 DAYS)

CBC: Hb: _____ ALT: _____ AST: _____

Urea: _____ Creatinine: _____

E) HOSPITAL CARD

National Medical Centre

OUT PATIENT DEPARTMENT

Date _____ OPD No. _____ MR # _____ Name of the Company _____

Patient's Name _____ Referred by _____

/o _____ Age _____ Sex _____

Address _____

_____ Phone _____

Consultant _____

In Patient No. _____ D.O.A. _____ D.O.D. _____

Weight in Admission _____ Weight in Discharge _____

Prov. Diag. _____

Final Diag. _____

Biopsy _____

O.P. Procedure _____

DATE	COMPLAINT / PHYSICAL EXAM.	TREATMENT

F) TURNITIN PLAGIARISM REPORT

PEPTIC ULCER

ORIGINALITY REPORT

7%	5%	7%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	worldwidescience.org Internet Source	1%
2	link.springer.com Internet Source	<1%
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